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PENICILLIN COMES TO THE CORNER PHARMACY

THIS month, when public pharmacists have just begun to dispense penicillin, the JOURNAL presents a timely and comprehensive review of present knowledge on penicillin in a series of papers written especially for pharmacists by leading authorities. It is hoped that this issue will serve as a reference handbook and guide through which pharmacists may dispense penicillin wisely and discuss intelligently with their physicians the questions that will arise now that this new and remarkably effective antibiotic is available in every community for the first time. Here has been condensed from scores of sources the pertinent data of value and interest to the pharmacist, including considerable information not heretofore available.

Current reports indicate that effective oral forms of penicillin may not be far off. Purer forms of penicillin are becoming available which are expected to permit further extension of the dating period and may presage a form of penicillin that need not be refrigerated. The price of penicillin has already been reduced drastically during the past year, and if current efforts to synthesize the product are successful, this most widely effective agent in the pharmacist's armamentarium may also be among the least expensive. In the future, as now, pharmacists in all branches of the profession will play an important part in making penicillin readily available in effective forms which will save the lives of those who would be doomed by otherwise fatal infections.—*Ed.*

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PENICILLIN

OPPORTUNITY *AND* OBLIGATION

by **ROBERT P. FISCHELIS**

SECRETARY, AMERICAN PHARMACEUTICAL ASSOCIATION

THE advent of penicillin distribution through retail pharmacies brings to a climax the first experiment covering the introduction of a new drug under completely controlled conditions. It therefore poses certain new problems for the pharmaceutical profession. The solution of these problems will set a pattern for future activities. It is to be hoped that no missteps will be made and that manufacturers, wholesale distributors and dispensing pharmacists will rise to the responsibility of maintaining a voluntary distribution control over this drug which will prevent its misuse.

In this issue of the JOURNAL, as well as in the *Scientific Edition* for this month, authoritative and up-to-the-minute basic information on the production and use of penicillin is presented.

Penicillin had definitely proved its value in a long list of specific ailments before it was given a "new drug" status under the Food, Drug and Cosmetic Act. Until now it has been available only for those cases in which its therapeutic effectiveness has been established. It has been distributed only to depot hospitals where it could be administered or from which it could be supplied to other hospitals. In short, penicillin has been introduced and used under ideal conditions and without the customary commercial promotion.

Now comes the availability of this drug through the customary industrial channels with restrictions as to use and availability withdrawn. There is definite anticipation in some quarters that uneconomical, unscientific and wasteful use of this valuable remedy may occur. This is accentuated by the recent announcement of successful experimental use of penicillin by oral administration. The distribution problem thus becomes more complex because the general public use of the drug has heretofore been limited to parenteral injection, which required the services of a physician. Will it now be possible to buy penicillin pills, tablets, ointments or solutions over the counter and will penicillin become an ingredient in a variety of other pills and potions and will there be any control over its sale for oral administration? These are questions of grave importance.

Apparently penicillin is not a "dangerous" drug, since its effects are reported to be generally nontoxic. However, idiosyncrasies and individual reaction may contraindicate its use. Such legal control as there will be over its use and sale without medical advice must apparently come through the enforcement of the misbrading clause of the Federal Food, Drug and Cosmetic Act. One can hardly conceive of "adequate directions for use" on the label of penicillin for all of the infections in which it may be helpful. Consequently, the directions would have to be supplied by a physician in the first instance. This makes it a prescription product and takes it out of the class of over-the-counter products sold for self-medication.

Pharmacists are inquiring whether it would be permissible for them to compound prescriptions calling for the withdrawal of penicillin from ampuls intended for parenteral use and incorporating such penicillin into ointment vehicles and perhaps other bases for external use. It is important to bear in mind that while prescriptions are exempt from the application of certain regulations under the Food, Drug and Cosmetics Acts of the United States and of the several states, the safety of the patient must always be the prime consideration.

If a given physician knows exactly what his patient requires, and directs a pharmacist to compound a special formula based upon his prescription, the pharmacist is of course expected to use his professional knowledge and skill to provide the remedy required by the physician. But, just as the physician must be definitely sure of the proper application of penicillin in the specific

case for which he is prescribing, so the pharmacist must be sure of his procedure in dispensing this drug. This involves a knowledge of the keeping qualities of the drug, the extent to which its incorporation in certain vehicles or bases may inhibit its action, the keeping quality of the finished product and its consequent effectiveness at the time of use, and the possibility of reactions by the sensitive or sensitized patient.

These factors become even more important when a pharmacist attempts to prepare special formulas or prescriptions, in which penicillin is an ingredient, for distribution to other pharmacists. As a distributor of a prescription product he must supply proper labeling, including appropriate and necessary warnings with respect to stability, use and dosage. He must assure himself that the product he is distributing meets the "new drug" requirements of the Federal and state Food, Drug and Cosmetics Acts. In other words, he assumes the full responsibility which any manufacturer must assume under the provisions of Federal and state laws governing the quality, safety and commerce in new drugs.

Apparently there is an opportunity for the entire drug industry to demonstrate in the future marketing of penicillin that it views the production and dispensing of such drugs as a health service which entails all of the responsibilities devolving upon the health professions as a group.

If producers and wholesale distributors can refrain from marketing the drug in any but medically approved form, and if the retail dispensers of drugs can desist from making the drug available except on prescription, until more promiscuous use is entirely safe and not wasteful, the profession will meet an important test of its ability to function in the true interests of the public health. If retail dispensers will handle this product only when they are prepared to properly store it at refrigerator temperature and will watch expiration dates and other warnings and cautionary statements on labels its expert and safe dispensing is assured.

PENICILLIN STABILITY PERMITS INCREASED PERIOD OF DATING

The dating period of nearly all brands of penicillin has now been extended to twelve months at temperatures below 10° C. as a result of studies by the Food and Drug Administration, reported in the April *Scientific Edition* of THIS JOURNAL, and data supplied by manufacturers.

After one year, commercial penicillin sodium stored in sealed ampuls at 8° C. showed "no significant loss of potency. At 25° C. and 37° C. some lots remained stable while others lost considerable activity. At 56° C. all samples lost considerable potency. Crystalline penicillin sodium held for one month at 56° C. or heated for two hours at 100° C. shows no detectable loss of activity."

During the experiments it was noted that especially marked deterioration occurred when moisture content exceeded 4%.

Stability tests on the magnesium, lithium, potassium, strontium, calcium and ammonium salts of penicillin gave results similar to those obtained for the sodium salt, with the exception of the ammonium salt which lost all potency within three weeks at 56° C.

Penicillin of recent manufacture from 17 different producers was found to remain stable for one month at 37° C. (98.6° F.).

Drs. W. A. Randall, H. Welch and A. C. Hunter, who reported the study, conclude that "penicillin sodium is a relatively stable product when it is kept below 10° C., and when properly prepared will maintain its potency for at least one year under such temperature conditions. Even at higher temperatures short periods of exposure should have no deleterious effect on the potency."

BETTER VEHICLE IS NEEDED FOR ORAL USE OF PENICILLIN

To obtain serum concentrations of penicillin after oral administration comparable to those obtained after intramuscular injection about five times as much penicillin is required. This is the conclusion of workers at the Cornell University Medical College and the New York Hospital after tests on four different methods of ingestion (*Science*, 101: 228, 1945).

The preparations administered were: (1) penicillin in corn oil, (2) penicillin in water, (3) penicillin in water preceded by a buffer, magnesium trisilicate, and (4) penicillin in peanut oil and 4% beeswax. The concentrations of penicillin in the blood serum produced by the four different methods were all of the same order of magnitude.

The action of the penicillin appeared to be prolonged, however, by use of the oil and the beeswax and oil as vehicles.

PENICILLIN

PROPERTIES AND STANDARDIZATION

by HENRY WELCH, ROBERT P. HERWICK and ALBERT C. HUNTER

FOOD AND DRUG ADMINISTRATION

INFORMATION ON UNITAGE AND DRUG FORMS PRESENTED FOR PHARMACIST; INCREASED STABILITY AND PURITY ACHIEVED BUT PRETESTING BY FDA IS PLANNED TO IMPROVE CONTROL

THE story of American penicillin production, related elsewhere in THIS JOURNAL, is outstanding even for a country known for its ability for doing things in a "big way." However, no one agency or manufacturer can be said to be largely responsible since it has been the combined efforts of a number of manufacturers, a number of agencies and individuals who have made it possible to supply great quantities of this highly valuable therapeutic agent to the armed forces of this country.

Much of the stimulation to production has been the result of well-planned and carefully done scientific investigations which have shown the need for penicillin. Probably never in the history of medicine has such progress in the evaluation of a drug been made in such a short time. Again the cooperative efforts of the best scientific minds in the country, planned and arranged by the Committee on Medical Research of the Office of Scientific Research and Development, have shown the efficacy or lack of it in the use of penicillin for the treatment of a great variety of diseases.

In September, 1943, at the request of our armed forces and the War Production Board, the Food and Drug Administration undertook the testing of each lot of penicillin produced in this country for potency, sterility, toxicity, pyrogenicity and moisture content.

During the early commercial production of penicillin an expiration date of three months when kept below 10° C. seemed advisable. Actually the three months' dating period was an arbitrary one since at that time little was known about the stability of this product. Because of this outdating period the prompt dispatch of penicillin to war areas where it was needed created an acute transportation problem, particularly when large quantities were needed overseas.

One of the first problems, therefore, was a study of the stability of penicillin sodium in order to determine whether it would be possible to increase the dating period. The Food and Drug

TESTING TOXICITY.—A sample of penicillin is injected into the tail vein of a mouse in the procedure for determining the safety of the drug.

Administration[1] has studied the stability of each manufacturer's product and it has been determined that penicillin sodium is a relatively stable drug. When kept at temperatures of 10° C. or below it will invariably maintain its activity for at least twelve months.

In fact, some evidence now available indicates that the penicillin of certain manufacturers should maintain its activity for at least eighteen months. This is in line with the known facts concerning the stability of crystalline penicillin sodium which, as far as is known, is stable indefinitely.

When penicillin was first produced in this country the purity and hence the potency were quite low. It was not uncommon in the assay of manufacturers' products to find potencies in the range of from 100 to 200 units per milligram. During the past year this situation has gradually changed so that at the present time the penicillin of most manufacturers has a potency in excess of 300 units per milligram. Consideration is being given to a requirement that penicillin have a purity of not less than 500 units per milligram. Many of the manufacturers today are producing penicillin at averages of from 700 to 900 units per milligram.

As the purity of penicillin is increased there is a gradual change in its color and consistency. The early crude material usually consisted of a dark brown amorphous powder. As the purity is increased the color changes to light brown, then yellow, until it finally reaches a pure white crystalline form.

However, there is no correlation between the color and the therapeutic efficacy of penicillin. Even the low potency (100 units per milligram) material was found to be just as efficacious unit for unit as the pure crystalline salt.

Physical Appearance Varies

Because of the different methods of drying or "lyophilization" of penicillin, which is an essential step in its production, the product takes several different forms in the ampul. Some manufacturers "shell" freeze their product before drying under vacuum. This results in an ampul in which the penicillin is a yellow to brown powder that clings to the sides of the ampul and is broken free only with difficulty. Other manufacturers "slab" freeze and dry their product which results in an ampul containing a dried mass of penicillin clinging to one side. If the ampul remains upright during the drying process the result is a relatively compact "button" of penicillin on the bottom of the ampul.

In all cases, regardless of the method of drying, the penicillin is readily soluble in distilled water, physiological salt solution, or glucose solution.

Uniform Packaging

At the present time, penicillin is dispensed quite uniformly in 25-cc. rubber-capped vials with an aluminum seal. Each of these vials contains the equivalent of 100,000 units of penicillin activity. All the vials carry on their labels the expiration date and directions for storage. Until such time as more data have been collected on the stability of penicillin at higher temperatures, the labels will carry a warning to maintain penicillin in the refrigerator at 10° C. (50° F.) or lower.

There is evidence to show that some penicillin sodium is stable when stored at even higher temperatures.[1] In general the greater the purity of penicillin sodium the greater is its stability, although there may be some exceptions to this rule. Certainly crystalline penicillin sodium is highly stable, withstanding temperatures of 100° C. for two hours or more.

Practically all of the penicillin now produced carries an expiration date of twelve months from the date of preparation. This does not mean that all the penicillin activity has been depleted by the end of the twelfth month. We have recently examined penicillin transported abroad without refrigeration and returned to the Administration for further examination after fifteen months. This material maintained its original potency.

At the present time there are at least three forms of penicillin, which have been designated "F," "G" and "X," and are referred to by the British as I, II and III, respectively. These penicillins differ in chemical structure, potency and stability. For example, penicillin "F" is much less stable than either penicillin "G" or "X." The commercial penicillin produced in this country is largely the "G" (II) penicillin.

Recently, considerable interest has been aroused in the "X" form, so-called allo-penicillin, because of its apparent greater effectiveness against certain microorganisms.[2] Preliminary clinical studies indicate that it is effective in the cure of gonorrhea with a single intramuscular injection of 25,000 units, as compared to 100,000

units of commercial penicillin of type (G).

The labeling of ampuls of penicillin as containing 100,000 units means that they contain 100,000 units of activity as measured by an *in vitro* assay. These "units" are for all practical purposes the same as the original "Oxford" unit.

The Oxford unit, described by the Oxford group in England,[3] was that amount of a partially purified solution of purely arbitrary strength which, when diluted in phosphate buffer saturated with ether, resulted in a zone of inhibition of 24 mm. diameter when tested by the cup-plate method. The Oxford group adopted as the arbitrary unit that amount of penicillin which, when dissolved in 1 cc. of water, gave the same inhibition as the standard. Later, for the benefit of those who used the series dilution method of assay, the Oxford unit was defined as that amount of penicillin which, when dissolved in 50 cc. of meat extract broth, just inhibited completely the growth of the test strain of *Staphylococcus aureus*.

Obviously, the use of such an arbitrary unit as a standard, wherein dependence would be placed on each individual laboratory to determine for itself what its unit would be on the basis of the above, involved a number of variables which could only be resolved by the development of some standard of reference.

The responsibility for the sponsorship of a penicillin standard in this country was placed upon the Food and Drug Administration. Fortunately with the preparation of crystalline penicillin sodium it became possible to create a master standard consisting of the chemically pure sodium salt. Because of the scarcity of this material it was not possible to distribute the master standard for general use. It was therefore necessary to develop a secondary or reference standard with the value determined by assay against the master standard.

Early in 1944 this Administration set up as the primary standard pure crystalline penicillin sodium, and it was given a value of 1650 units per milligram. Thus, an ampul of the commercial salt of penicillin containing 100,000 units would contain the antibiotic activity of approximately 60 milligrams of crystalline penicillin sodium. The reference standard, which is distributed to manufacturers and other responsible groups, consists of a calcium salt of penicillin with an assigned value of 370 units per milligram based on the master standard.

In October 1944, at the International Conference in London, crystalline penicillin sodium "G" (II) was accepted as the primary standard. The

TESTING PENICILLIN FOR PYROGENS.—A sample of penicillin is dissolved in pyrogen-free, double distilled water. The solution is then injected into the marginal vein of the rabbit's ear. Rectal temperature is taken before injection and at one, two and three hours following injection. If the increase in temperature in a group of test animals is greater than the official limits, the lot must be reprocessed.

unit was defined by international agreement as the specific penicillin activity contained in 0.6 microgram of this primary standard. Thus, one *milligram* of the International Standard is equivalent to 1667 units. The International Conference further accepted as a working standard the calcium salt reference standard of the Food and Drug Administration. One unit of this standard (penicillin calcium) is contained in 2.7 micrograms, and thus one milligram is equivalent to 370 units. The method of assay now in use by the Food and Drug Administration for determining the potency of penicillin is given below.

Method of Assay for Potency

Cylinders (Cups): These are made from standard wall pyrex or from porcelain, stainless steel, or aluminum tubing of the same thickness (±0.1 mm.) 1 cm. long and 8 mm. ± 0.1 mm. outside diameter. The 1-cm. lengths are, with the exception of the stainless steel cylinders, beveled inside on one end at an angle of 30° to 40°. After beveling, the end surface is ground with a fine-grade abrasive to provide a smooth edge.

Standard Penicillin: The primary standard is crystalline penicillin sodium "G" (II). The unit of penicillin has been defined by international agreement as the specific penicillin activity contained in 0.6 microgram of this primary standard.

Reference Standard: A working standard of penicillin calcium has been adopted, 2.7 micrograms of which are equivalent to one unit, *i. e.*, 1 mg. is equivalent to 370 units.

The standards are kept in tightly-stoppered vials which in turn are placed in larger stoppered tubes containing "Drierite" (anhydrous $CaSO_4$). The standards are kept constantly in the freezing compartment of an electric refrigerator. Every three days between 4 and 5 mg. of the working standard is carefully weighed out and diluted with sterile 1% phosphate buffer (*p*H 6) to provide a stock solution containing any convenient concentration. The daily working dilution is then made from this stock solution.

Preparation of Samples: All samples are first diluted aseptically with pyrogen-free distilled water to contain 4000 units per ml. For use in the assay for potency, 1 ml. of the 4000-unit-per-ml. solution is placed in a 100-ml. volumetric flask and made up to volume by the addition of sterile distilled water. One milliliter of the 40-unit-per-ml. solution is transferred to a flask containing 39 ml. of 1% phosphate buffer *p*H 6. This buffer solution of the penicillin containing 1 unit per ml. is then further diluted to make a solution containing 0.25 unit per ml. by adding 1 ml. of the 1-unit-per-ml. solution to 3 ml. of buffer. These last two dilutions are used in the assay for potency.

Preparation of Plates: Plates are prepared by adding to each Petri dish (20 x 100 mm.) 21 ml. of agar. After the agar has been distributed evenly in the plate and has hardened the plates are stored in the refrigerator until the following day or may be kept several days before being used. The test organism, *Staphylococcus aureus* (F.D.A. 209-P), is maintained on agar slants and transferred to fresh agar about once a week. On the day preceding the assay the culture is transferred from the agar slant into broth. The next day 2 ml. of this 16–24 hour broth culture is added to each 100 ml. of agar which has been melted and cooled to 48° C. The culture and agar are thoroughly mixed and 4 ml. added to each of the plates containing the 21 ml. of the uninoculated agar. The plates are tilted back and forth to spread the inoculated agar evenly over the surface. The glass covers of these inoculated plates are now replaced by porcelain covers. The cylinders are placed on the agar surface by dropping them through the openings of a plastic cylinder guide having 4 openings.

Assay for Potency: For each sample four plates are used. One cylinder on each plate is filled with the 1 unit standard and one with the 0.25 unit standard. The unknown is added in estimated dilutions of 1 unit and 0.25 unit to the remaining 2 cylinders on each plate. The plates are carefully placed in racks and incubated 16–18 hours at 37° C. After incubation the diameter of each circle of inhibition is measured to the nearest 0.2 mm. using a Quebec colony counter with a millimeter scale etched into the supporting glass over the light source. The potency is computed by comparison of the diameters of the zones of inhibition of the unknown with those of the standard.

Penicillin is effective against gram-positive organisms, but in general has little or no effect on the gram-negative types. This does not include the gram-negative diplococci such as the meningococcus and gonococcus which are known to be quite sensitive to penicillin. Excellent reviews of the clinical use of penicillin have been published.[4–7]

The fact that at the present time practically all penicillin is given parenterally and that it is not effective against certain types of gram-negative organisms makes it mandatory that it be dispensed as a sterile product and handled aseptically.

Penicillin is one of the most innocuous drugs known since for all practical purposes it may be considered to be atoxic. Extremely large quantities may be injected in man without ill effect.

It is true that some of the early batches of penicillin had some toxic properties, but with increase in purity (potency) these toxic effects disappeared.

The very nature of the manufacturing process, however, precludes the possibility of avoiding the production of pyrogens and other toxic substances since during the growth of *Penicillium notatum* there is lysis of the mold mycelia and spores. Such lysis releases into the surrounding medium the toxic and thermogenic materials. The processes of extraction are such that both pyrogens and toxic substances are partially removed and the use of adsorptive filtration, with either Seitz or Ertel filters, or the equivalent, when properly carried out, sterilizes the product and eliminates those pyrogens not removed by the extraction process.

Early in 1944 the Food and Drug Administration studied the acute toxicity of over 300 lots of commercial penicillin produced by the 14 manufacturers[8] in production at that time. Although it was shown that there was a wide variation in the acute toxicity of the penicillin tested by injection in mice, all lots passed the so-called mouse "safety test." This safety test is conducted by injecting each of 5 mice intravenously with 2000 units of the penicillin. No deaths should occur within forty-eight hours. If any animal dies the test is repeated with unused mice, and if all animals survive the repeat test the product is considered satisfactory.

Pursuing further the investigation of the toxicity of penicillin sodium, 60 lots from 10 manufacturers were studied to determine their ability to inhibit phagocytosis. A definite correlation between the toxicity of the penicillin preparations and their potency was demonstrated.[9]

During an investigation of the toxicity of various salts of penicillin it was noted that penicillin calcium showed a greater toxicity than penicillin sodium when both were produced from a single master lot. Although this greater toxicity should not discourage its clinical use, the investigation did indicate that the toxicity penicillin calcium does have is associated with the cation used in its preparation. Other studies involving seven salts of penicillin (sodium, lithium, ammonium, strontium, calcium, magnesium and potassium) made from a single master lot have shown that the toxicity of all these salts of penicillin is primarily due to the cations used in their preparation.[10, 11] All the seven salts have been used clinically and preliminary studies indicate that they are equally effective.

Both the Army and the Navy have reported that certain lots of commercial penicillin sodium produced severe pain on intramuscular injection. In some cases repeated injections were followed by the formation of sterile abscesses containing insoluble material with no antibacterial activity.

From studies made on intramuscular irritation with several different salts of penicillin[12] it appeared that the site of injection was determinative to a certain extent of the degree of pain produced, and furthermore that the pain was usually produced with low potency material. Later studies[13] showed that there was a significant correlation between the purity (potency) of commercial penicillin sodium and irritation following intramuscular injection in man. As the potency was increased in units per milligram there was a corresponding decrease in the pain produced. Evidence was elicited that of the three sites of injection tested (buttocks, triceps and deltoid) the least pain resulted from injection into the buttocks. There was a marked decrease in the pain produced in man when the potency of the product injected was 700 units per milligram or more. It seems fair to assume that with an increase in purity of penicillin as production is further improved little or no pain following intramuscular injection should be expected.

There have been a limited number of reports of mild toxic symptoms following clinical use of penicillin sodium. As reported by Keefer[14] and Lyons[15] the commonest single complication is urticaria. The suggestion has been offered that these sensitization phenomena were probably due to impurities in the commercial preparations. However it was later shown that sensitivity could be demonstrated not only with commercial penicillin sodium, but also with crystalline penicillin.[16] Other reactions reported and thought to be due to impurities are chills (with or without fever), headache, faintness and flushing of the face, unpleasant taste after injection, muscle cramps and thrombophlebitis following intravenous injection. However, during eighteen months of penicillin therapy, no significantly harmful effects have been observed.

During the war emergency the control of penicillin has been handled through an arrangement between the Food and Drug Administration, the War Production Board, and the Army and Navy. Now that penicillin is available for general dis-

tribution to civilians, some control measures will be needed to handle this important therapeutic agent. At the present time plans are under way to request an amendment to the Federal Food, Drug and Cosmetic Act to require pretesting and certification of all lots of penicillin before distribution in interstate commerce.

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PENICILLIN

BIOLOGIC CHARACTERISTICS

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DIVISION OF MEDICINE
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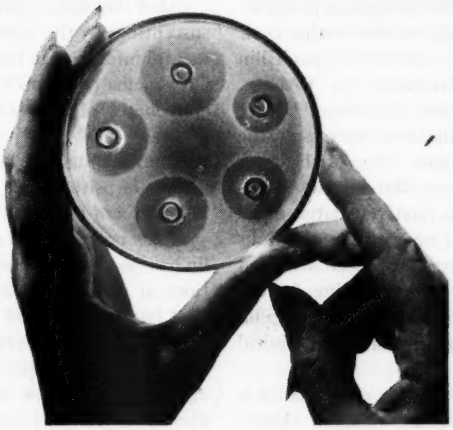
SLOW ACTION OF PENICILLIN IS IMPORTANT FACTOR IN THERAPY; ACTIVITY IN BODY FLUIDS GIVES MARKED ADVANTAGE OVER OTHER ANTIBACTERIAL AGENTS BUT MANY ORGANISMS ARE NONSUSCEPTIBLE

FOR a period of ten years after its discovery by Fleming,[1,3] penicillin was of interest to the bacteriologist as a substance which would prevent the growth of certain kinds of bacteria and permit the growth of others. It was used in bacteriologic media for cultivating organisms such as the influenza bacillus which ordinarily would be overgrown by other bacteria present in sputum and in other specimens sent for bacteriologic examination. In 1940 Florey and other investigators at Oxford announced the successful purification of penicillin and described its behavior under various experimental conditions.[4] Publications by the investigators[5] at Oxford and the original observations by Fleming provided a broad understanding of this new substance and an accurate evaluation of its probable worth as a therapeutic agent. The observations of the British scientists have been confirmed by many investigators.

In spite of the vast amount of work that has been done with penicillin, it is not known precisely how penicillin acts against bacteria. Although there is some evidence that penicillin can be bactericidal under certain conditions, the opinion most widely held today is that penicillin is primarily bacteriostatic.

Gardner[6] described striking changes in the appearance of bacteria after they had been exposed to penicillin. Spherical forms would swell to many times the normal size and, in some instances, susceptible rod-shaped bacteria would become ten or more times as long as normal. Gardner suggested that these changes were due to failure of the cells to divide. The bacteria continue to increase in size but multiplication does not take place.

Hobby and Dawson[7] have shown that conditions which increase the rate of growth of bacteria increase the rate at which penicillin acts and that the effect of penicillin against bacteria is decreased by conditions that decrease the rate of bacterial growth. This work, as well as that of Gardner, indicates that penicillin acts on bacteria at the time they divide. It should be reemphasized that the exact way in which penicillin acts to prevent normal bacterial multiplication is yet to be discovered.



THE CUP TEST for potency is made by placing dilutions of standard and unknown penicillin in small cups or cylinders on the surface of agar inoculated with Staphylococcus aureus. After incubation for 16–18 hours the diameter of each circle of inhibition is measured. The size of the area in which bacterial growth has been checked by the unknown, compared with that produced by the standard, indicates the potency.

On the other hand, a great deal has been learned about the biologic characteristics of penicillin. The results of numerous studies, beginning with Fleming's first description of the substance,[1] indicate that penicillin has many peculiar properties of its own that sharply distinguish it from ordinary germicides. The properties of purified penicillin had to be determined by Florey and his co-workers at Oxford[4] before they attempted to use the substance in treatment of patients. From the description, given by the Oxford investigators, of the behavior of a fairly pure penicillin it became apparent that, taken as a whole, the features which distinguished penicillin from other antiseptic preparations combined to produce a therapeutic agent of unique excellence. Some of its distinguishing features should be kept in mind if the drug is to be used to advantage.

In the first description of penicillin, Fleming pointed out that it is a slow-acting antiseptic substance. Unlike germicides which kill bacteria in a few seconds or minutes, penicillin sometimes requires hours to act on susceptible bacteria. Increasing the amount of penicillin may somewhat shorten the time required for it to act on a susceptible culture but there is usually a limit to the increase in effectiveness of the drug after a certain concentration has been reached.

The fact that penicillin requires a certain amount of time to act on bacteria is very important from the standpoint of therapy. If penicillin is to be used as a local agent, it must be kept in contact with the area of infection for at least several hours. When it is administered parenterally it is important to maintain an effective amount of penicillin in the blood stream or at the site of localized infection.

Fleming also pointed out that, unlike most other antiseptic preparations, penicillin is not toxic for animals. He observed that white blood cells were not injured when they came in direct contact with penicillin. The Oxford investigators[4,5] showed that after penicillin was purified, it still was not toxic for animals or for cells growing in tissue culture. They emphasized a remarkable characteristic of penicillin; namely, its ability to act on bacteria even in the presence of blood serum, body fluids and living cells. The presence of pus, which would neutralize the antibacterial action of the sulfonamides, did not interfere with the activity of penicillin.[5]

We were able to confirm all of these observations by studying the effectiveness of penicillin against bacteria in the presence of growing cells in tissue culture.[8,9] We also studied many other germicides by this method and found that their ability to kill bacteria was greatly diminished in the presence of serum.[10] Only a few showed any activity at all, and most of these were rather toxic for tissues. Even the relatively crude penicillin available at that time was from ten to fifty times less toxic by weight and many times more effective, against *Staphylococcus aureus*, than other germicides included in the test.

If similar tests were done with penicillin avail-

able at the present time, the differences probably would be even more striking because further purification has increased the activity of penicillin against bacteria and decreased its toxicity. Florey and Jennings,[11] and Robinson,[12] have shown that further purification decreased the toxicity of penicillin for mice. One of the most remarkable features of penicillin is that it has been given to patients in relatively large doses over a long period without toxic effects of serious consequence. Few reactions have been observed and these are thought to have been due, for the most part, to substances other than the penicillin present in the preparations available for clinical use.

Another distinguishing feature of penicillin is its selective action on certain bacteria. Fleming[1-3] found that most of the bacteria that were susceptible were gram-positive although some gram-negative cocci such as the gonococcus and meningococcus were also inhibited. On the other hand, not all gram-positive organisms were affected. As penicillin became more and more highly purified it was possible to use it against bacteria which had been relatively resistant to the crude preparations. The results of laboratory tests by the British workers[1-5] and a host of other investigators indicate that penicillin is effective against the following organisms under cultural conditions:

Susceptible Organisms

Diplococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius
micro-aerophilic streptococci
Staphylococcus aureus
Staphylococcus albus (some strains)
Neisseria gonorrhoeae (gonococcus)
Neisseria intracellularis (meningococcus)
Actinomyces bovis
Bacillus anthracis
Bacillus subtilis
Clostridium botulinum
Clostridium tetani
Clostridium perfringens (Clostridium welchii)
Clostridium septicum
Corynebacterium diphtheriae
Micrococci (some strains)
Streptobacillus moniliformis[13]
Erysipelothrix rhusiopathiae[14]
Hemophilus ducreyi[15]

Other infectious agents which have been found susceptible to the action of penicillin in experimental infections in laboratory animals are:
Treponema pallidum[16]
spirochetes of relapsing fever[17] and Weil's disease[18]
Spirillum minus (a cause of rat-bite fever)[19]
viruses of psittacosis[20] and ornithosis[21]
Rickettsia of typhus fever[22]

It has not yet been demonstrated that penicillin can be used successfully in treatment of infections of man due to all of the infectious agents that have been found susceptible under laboratory conditions. Further clinical experience is needed but it is gratifying that so many infectious agents found to be susceptible experimentally also have yielded to penicillin when it has been used in the treatment of infections of man.

Nonsusceptible Organisms

At the present time the following organisms are considered to be relatively insensitive to the action of penicillin:

colon-typhoid-paratyphoid-dysentery group of organisms
whooping-cough bacillus
Hemophilus influenzae
Proteus vulgaris
Streptococcus faecalis
Pseudomonas aeruginosa
Klebsiella pneumoniae
Vibrio comma
tubercle bacillus
abortus group of organisms (causing undulant fever)
pathogenic fungi
Toxoplasma
Trypanosoma equiperdum
pleuropneumonia-like organisms
viruses of influenza and poliomyelitis
malarial parasites

It should be emphasized that different strains of the same organism may vary greatly in their sensitivity to penicillin. This was pointed out by the Oxford investigators for different strains of pneumococci and also has been shown to be true of many other species of bacteria.

It is highly desirable to be able to determine the penicillin sensitivity of the causative organism in treating infections, particularly when the condition does not respond to penicillin therapy or

when the causative organism belongs to a group of bacteria likely to contain strains that are not sensitive to penicillin.[1, 23] For instance, many green-producing streptococci are not sensitive to penicillin. It has been shown clinically and in the test tube that sensitive strains may become resistant to the action of penicillin.[5] Disappointment in the clinical use of penicillin is likely to result if these possibilities are not kept in mind.

Destroyed by Penicillinase

Not only is penicillin unable to inhibit multiplication of certain kinds of bacteria but many resistant organisms actually are capable of destroying penicillin. Abraham and Chain[24] found that penicillin is destroyed by an enzyme called "penicillinase" which is present in the bacterial cells of certain organisms. When a solution of penicillin becomes contaminated with bacteria which produce penicillinase, all of its activity is lost within a short time.

It is best to use solutions of penicillin fairly soon after they are prepared or to keep them under refrigeration until they are used. When penicillin was first produced it was not as pure as it is now, as has been said, and when it was put in solution and kept in the refrigerator it would lose activity even if it was kept sterile. Most of the penicillin manufactured at present will retain its full activity in solution in the refrigerator for many days, provided it is not contaminated with bacteria. In the dry state, in the ampul, many samples of penicillin will retain their activity even when kept at room temperature for months but some products will lose some of their activity at room temperature. For this reason ampuls of penicillin are stored in the refrigerator. High temperatures rapidly destroy the activity of penicillin.

Other things capable of destroying penicillin must be guarded against in its manufacture. Some of these are acids, alkalies, oxidizing agents and reducing agents. The destruction of penicillin by acid has an important bearing on its pharmacology because gastric acidity destroys a large proportion of penicillin that is administered orally. Much effort is being made to solve this problem and to find a satisfactory way of giving penicillin by mouth. The problem is complicated by the fact that intestinal bacteria which are capable of destroying penicillin are particularly numerous in the colon. Penicillin is absorbed in the upper part of the small intestine, where intestinal bacteria are usually not numerous. If, however, it is so protected on oral administration that it can find its way into the colon or if it is introduced by rectum, it is destroyed in the colon before absorption can take place. This precludes its administration by the colonic route.

Another problem which is receiving the attention of investigators is how to maintain the desired amount of penicillin in the blood stream without resorting to frequent subcutaneous, intramuscular or intravenous injections. Penicillin is rapidly eliminated by the kidneys; consequently, methods of administration are chosen which will provide entrance of penicillin into the blood stream throughout the period of treatment. Although these problems are of pharmacologic importance, the advances that have been made will not be included in this discussion.

The ultimate place of penicillin in relation to other preparations, such as the sulfonamides and other antibiotic substances, will depend on a number of factors. As penicillin becomes more plentiful it probably will be the material of choice in certain diseases that now respond in a fairly satisfactory manner to the use of sulfonamides. On the other hand, many conditions that cannot be cured by penicillin alone may respond to the simultaneous use of penicillin and one of the sulfonamides.

Synergism Studies

Laboratory tests with penicillin in combination with one of the sulfonamides have given varied results, depending on the bacterial strains being tested. Some investigators have found that some of the sulfonamides increase the effectiveness of penicillin against some bacteria but not against others.[25–27] Other studies have not indicated a synergistic effect.[28] Further experience is necessary before conclusions can be formed but it is apparent from laboratory and clinical studies that when penicillin and one of the sulfonamides are used simultaneously, one does not interfere with the activity of the other.

Another antibiotic substance of microbial origin in general use for topical application is tyrothricin, a substance isolated by Dubos[29] from a soil bacillus. He found that it was toxic for animals when given by intraperitoneal or by intravenous injection. In this connection it has

been found that tyrothricin possesses marked hemolytic properties.[30] We found that the toxicity of tyrothricin for tissues was low compared with its ability to inhibit multiplication of certain pathogenic bacteria[9,10] and it has proved to be effective in the treatment of localized infections caused by organisms sensitive to its action.

Penicillin and tyrothricin are effective against many of the same bacterial species but multiplication of some bacteria is inhibited by one and not by the other.[8] Tyrothricin is more stable than penicillin in that it is not destroyed by bacteria and it retains its activity in solution for a long period. In experiments on bacterial inhibition by mixtures of penicillin and gramicidin (one of the active fractions of tyrothricin), we found that one did not decrease or enhance the activity of the other.[8]

Another antibiotic preparation of microbial origin which appears to be promising is streptomycin, a substance described by Schatz, Bugie and Waksman.[31] The substance was isolated from a strain of *Actinomyces griseus*. It is effective in test tube experiments against numbers of bacterial species, including many gram-negative organisms that are not sensitive to penicillin. It has been used successfully in the treatment of a number of experimental infections in laboratory animals[32–36] but reports of its clinical use have not yet appeared. It is to be hoped that, as time goes on, substances will be developed that can be used successfully in the treatment of conditions for which there is no satisfactory therapeutic agent at the present time.

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FDA PLANS TO CERTIFY ALL PENICILLIN BEFORE RELEASED

Certification of each batch of penicillin by the Food and Drug Administration as a permanent policy appears likely through legislation about to be introduced into Congress. This will take the form of an amendment to section 506 of the Act, which already provides for similar certification of drugs containing insulin.

If adopted, the measure will authorize continuance of the present system of control which calls for purity and potency tests by FDA on each batch before it is released for distribution to either civilian or military agencies.

For several months past, Food and Drug officials have been considering possible plans to assure continued high quality of penicillin after termination of wartime controls.

They foresee that the wide publicity that has been given to the effectiveness of penicillin will induce many additional manufacturers to enter the production field when War Production Board restrictions on equipment have been removed.

"The occasional mishaps in the best controlled establishments lead to the inevitable conclusion," the FDA recently stated, "that attempts to produce penicillin with inadequate equipment will at best result in the manufacture of products lacking the necessary potency, with the added possibility that toxic impurities may be present."

Since the effect of the drug often does not become manifest for twelve to eighteen hours after administration, the ineffectiveness of a low-potency penicillin might not be recognized until a critically ill patient is past hope of recovery.

In view of these possibilities, the certification plan was proposed and submitted to pharmaceutical organizations and manufacturing laboratories for comment. Little, if any, opposition has developed, and early passage of the amendment is expected.

REAMER NAMED TO BOARD

I. Thomas Reamer, chief pharmacist at Duke Hospital, has been named to the North Carolina Board of Pharmacy to fill the unexpired term of the late E. V. Zoeller.

Mr. Reamer, alumnus of the University of Maryland and former pharmacist at Johns Hopkins, is also secretary of the American Society of Hospital Pharmacists.

PENICILLIN PRODUCERS

Here is the latest available list of pharmaceutical and chemical laboratories engaged in the manufacture of penicillin:

ABBOTT LABORATORIES

BEN VENUE LABORATORIES, INC.

CHEPLIN LABORATORIES, INC.

COMMERCIAL SOLVENTS CORPORATION

CUTTER LABORATORIES, INC.

HEYDEN CHEMICAL CORPORATION

HOFFMANN-LA ROCHE, INC.

LEDERLE LABORATORIES, INC.

ELI LILLY AND COMPANY

McKESSON AND ROBBINS, INC.

MERCK AND COMPANY, INC.

PARKE, DAVIS AND COMPANY

CHAS. PFIZER AND COMPANY, INC.

SCHENLEY LABORATORIES, INC.

E. R. SQUIBB AND SONS

THE UPJOHN COMPANY

WINTHROP CHEMICAL COMPANY, INC.

WYETH INCORPORATED

There is also a rapidly growing list of companies serving as distributors of penicillin. As of March 13, these were:

George A. Breon & Company

Burroughs Wellcome & Company, Inc.

Harrower Laboratories, Inc.

G. F. Harvey Company

Lakeside Laboratories, Inc.

McNeil Laboratories, Inc.

Wm. S. Merrell Company

National Drug Company

Carroll Dunham Smith Pharmacal Co.

R. J. Strasenburgh Company

United Drug Company

Wm. R. Warner & Company

PENICILLIN

PRESENT-DAY PRODUCTION METHODS

by SCIENTIFIC AND ENGINEERING STAFFS, CHAS. PFIZER & COMPANY

THE PRODUCTION OF PENICILLIN HAS BEEN A WARTIME TRIUMPH FOR AMERICAN LABORATORIES; HERE IS HOW IT'S DONE, FROM THE MOLD ORGANISM TO THE FINAL PRODUCT

War in itself is essentially evil yet it has often acted as a stimulus toward developments of great value to humanity. An outstanding example of this is the mass production of penicillin; ordinarily this would have required a decade or more for its accomplishment, but in the present emergency only a fraction of that time intervened from the time research was begun until large plants were in actual operation.

Although the original discoveries were British, large-scale manufacture is a monument to American scientific genius. In this the executive, research, engineering and production staffs of about twenty manufacturers, the Northern Regional Research Laboratories of the Department of Agriculture, the War Production Board, the Office of Scientific Research and Development, the Food and Drug Administration, the Office of Production Research and Development and various universities under contracts with it, the National Research Council, the National Institute of Health, and other official and semi-official agencies have all cooperated.

Basically, present-day production of penicillin involves the following steps:

(1) Growth of the mold on a suitable medium. It is during this step that the drug is actually produced.

(2) Extraction of the penicillin from the medium and its purification by chemical means.

(3) Drying and packaging of the final product under aseptic conditions.

The relative instability of penicillin, particularly when in solution, introduces many complications into each of these steps.

In the laboratories of the manufacturers, the Northern Regional Research Laboratories, and the universities under contract with the O.S.R.D., a vast amount of research has been devoted to the development of satisfactory strains of molds for the production of penicillin. As a result of these studies, strains are now available which are particularly suitable for each type of fermentation process, the penicillin content of the resulting broths being many times as great as in the original experiments.

For its metabolic processes the mold used requires the presence of oxygen dissolved in the media on which it grows. Also contamination with other organisms generally results in the production of enzymes which destroy penicillin. Accordingly, it is necessary to grow the mold in the absence of other organisms and with sufficient exposure to sterile air so that enough oxygen is dissolved in the medium to supply that needed by the mold. The simplest method of accomplishing this is by growing it on shallow layers of medium contained in flasks or bottles closed with cotton so that air can enter but contaminating organisms cannot.

For large-scale production, however, this process requires the handling of an enormous number of flasks or bottles, so processes have been developed whereby the mold can be grown efficiently on large volumes of medium. One is the so-called "bran process" in which the medium consists of bran that has absorbed the other nutrient ingredients; growth takes place in shallow pans or large rotating drums.

A more commonly used process is called "submerged fermentation." The medium used, consisting of an aqueous solution of lactose, corn steep liquor and nutrient salts, is prepared in large closed tanks of several thousand gallons capacity. These tanks are provided with agitation, coils for heating or cooling, and aerators through which sterile filtered air can be bubbled through the solution.

The prepared medium is first heat-sterilized

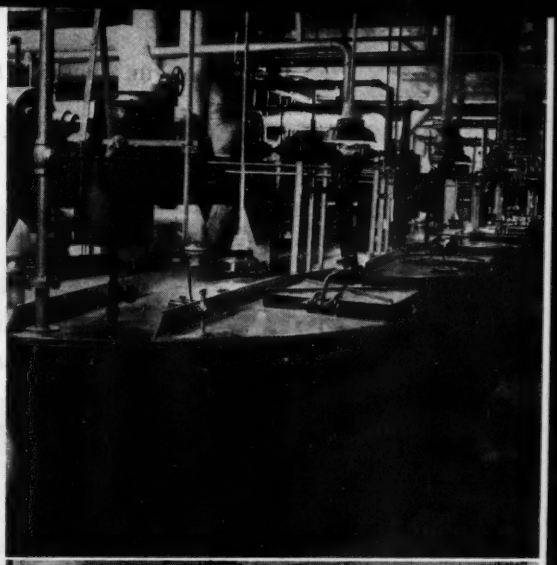
and then cooled to the proper temperature for the growth of the mold. It is next inoculated with a rapidly growing inoculum of the proper organism, strains of either *Penicillium notatum* or *Penicillium chrysogenum*, developed at the Northern Regional Research Laboratories and the University of Minnesota, being used in our process. With constant agitation and aeration growth of the mold takes place throughout the entire volume of nutrient broth.

During this growth penicillin is produced and secreted in minute amounts into the medium. At the same time, however, because of its instability when in solution, the penicillin produced is being partially destroyed, and as its concentration increases the amount of destruction in unit time also increases. Eventually a point is reached at which the rates of production and of destruction are equal and if the operation is continued beyond this point destruction exceeds production and the concentration drops.

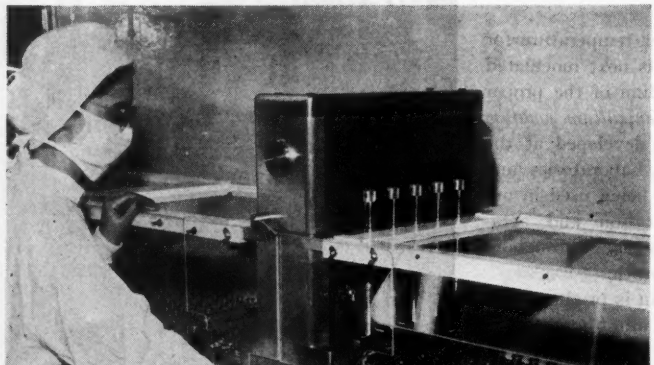
It is evident that fermentation should not be allowed to proceed beyond the optimum time for maximum production, so the concentration of penicillin in the broth is determined at frequent intervals, using a rapid turbidimetric method of assay developed in the Pfizer laboratories.

Like most natural processes, the growth of the mold produces heat. Since it is essential that the temperature be controlled within very narrow limits, this heat must be removed by the cooling coils. An elaborate system of automatic control has been installed, by which this is accomplished.

When, after several days, fermentation has proceeded to the optimum point, the mycelia resulting from the growth of the mold are removed by filtration and washed free of the penicillin-containing broth. The combined filtrate and washings are now ready for the extraction and



SUBMERGED GROWTH OF MOLD occurs in tanks of several thousand gallons capacity. The tops of the tanks, which contain a corn steep medium inoculated with penicillium, are shown in the upper photograph. CENTRIFUGAL SEPARATORS (center) are used during the extraction process to separate the water-immiscible solvent from the aqueous phase containing the penicillin. VIALS are washed and inspected (bottom) preparatory to sterilization before being filled with the concentrated purified penicillin solution.



FILLING MACHINE (left) measures penicillin solution into the vials at the rate of 10,000 vials per hour.

purification of the penicillin, the next step in the process. Considering that the concentration of penicillin in this broth only amounts to a few parts per 100,000 and that it is relatively unstable in solution, it is easy to visualize the difficulties encountered in developing a satisfactory process.

The first step consists in the adsorption of the penicillin on activated carbon by agitation of a suspension of carbon in the broth. The carbon is then filtered out by means of a plate and frame filter press and washed free of broth. Next the penicillin is removed from the carbon by elution with 80% acetone.

The acetone is then removed from this solution by means of a water-immiscible solvent, semiautomatic centrifugal separators being used to separate the aqueous phase, which contains the penicillin, from the solvent phase. Although the concentration of penicillin in the solution resulting from these two steps is some ten times as great as in the original broth and the greater part of the other substances originally present have been eliminated, further concentration and purification are still necessary.

The solution is first cooled to 0° C. and is then carefully acidified to *p*H 2, thus releasing the free acid of penicillin. This free acid is considerably less stable than are the salts of penicillin, hence the need for carrying out the operation at a low temperature and as rapidly as possible. This cooling and that required during fermentation and during subsequent steps of the extraction process have necessitated the installation of refrigeration equipment equal in capacity to that of a sizable ice plant.

The free acid is soluble in many water-immiscible solvents and the next step is its extraction from the aqueous solution by means of one of these solvents. The process we have developed is largely continuous. Automatic controls regulate *p*H and flow rates; and the solvent phase, containing the penicillin, is separated from the aqueous phase, in which most of the impurities remain, by a battery of centrifugal separators. Speed of operation and accurate control of *p*H and temperature are highly important during this step.

Next the penicillin is extracted from the solvent by means of a concentrated solution of sodium bicarbonate. Here, too, exact control of *p*H, temperature and flow rates is required, and equipment similar to that for the solvent-extraction step is utilized. As a result of these two operations the concentration of penicillin in the resulting solution is increased a further fifty to one hundred times over that of the previous solution and the greater part of the impurities present in the latter are eliminated. This completes the extraction process, and the solution, after filtering to remove pyrogens and possible organisms or spores, is ready for the drying operation.

Storage, recovery and rectification of the large volume of solvents handled require underground tanks, stills, columns, condensers, receivers and other distillation equipment. Because of the inflammability of these solvents every possible precaution must be taken to eliminate the fire hazard; storage tanks are underground, all

electrical equipment is explosion-proof, all lights are in vapor-proof globes, and a complete sprinkler system has been installed.

At present nearly all of the penicillin used is administered by injection as a solution, therefore it is of the utmost importance that the final product be sterile and free from pyrogenic and toxic substances. Because of its instability toward heat, penicillin or solutions of it cannot be sterilized by this means; therefore in subsequent steps any possibility of contamination must be rigorously avoided. Even in a laboratory this is not an easy matter and the accomplishment of this in large-scale production has required a very large amount of thought and effort.

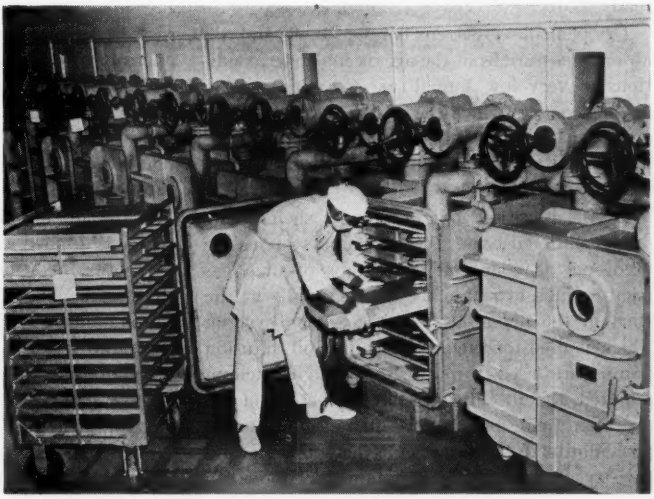
The final concentrated, purified solution of penicillin, which has been filtered through special asbestos pads to adsorb pyrogens and through a bacterial filter to remove any possible organisms or spores present, is first assayed for penicillin content by the Oxford cup method. As a check it is held for two days in refrigerated storage and assays are made on both days. These results are also checked by the turbidimetric method, although the results from the Oxford cup test are taken as official. From the value obtained the volume of solution required to supply 100,000 units is calculated. At the same time careful tests are made to assure that this solution is free from pyrogens and toxic substances.

After completion of these tests the solution is then accurately measured into the vials in which it is to be frozen and dried. These vials before use are washed a number of times, the final washings being with pyrogen-free, distilled water. This water is freshly distilled and is tested to assure the absence of pyrogens before being used.

After washing, the vials are inspected to be sure that they are in perfect condition, and they are then placed in specially constructed trays. These resemble a honeycomb and are bottomless except for small projections on which the vials rest. The covers to these trays provide an air space over the tops of the vials and overhang the trays, thus allowing room for the escape of vapor during the drying operation. Except while the vials are being filled, these covers remain on until the penicillin is dry and ready to stopper.

The trays of vials are next sterilized in live steam under pressure. The sterilizers have doors on opposite ends, one opening into the room in which the vials are washed and the other into the "sterile area," in which subsequent operations are carried out. In this area every effort is made to prevent the possibility of air-borne contamination: scrupulous cleanliness is maintained; the air entering is filtered and passed over a battery of ultraviolet lamps, and sanitizing lamps of this type are placed at frequent intervals and in strategic locations throughout all of the rooms. All operators are required to wear sterilized surgical gowns, caps, masks, gloves and shoe covers. Bacterial counts on the air in all rooms are made at frequent intervals to be sure that these precautions are functioning properly.

After sterilization the trays of vials are allowed to cool in a room in the sterile area set aside for this purpose, and representative samples are tested to be sure that they are pyrogen-free.



VACUUM DRYERS (right) are being loaded with trays of vials of penicillin solution. The finished product will contain not more than 1% moisture.

From here the trays pass to a multiple filling machine.

Each filling needle is set to deliver the proper amount of penicillin solution, calculated from the assay to contain 100,000 units (in practice a 4% excess is used), and is accurate to within 0.05 cc. Ten vials are filled at a time and the machine will fill at the rate of 10,000 vials per hour.

Before entering this machine the solution is filtered through fritted glass to remove any fibres. Since it is necessary to uncover the trays during filling, extraordinary precautions are taken to prevent any possible contamination —the operation is carried out in a small room supplied with specially filtered, sterilized air; the walls and floors are washed down at frequent intervals with an antiseptic solution; as mentioned above, the operators wear sterilized caps masks, gowns, gloves and shoe covers; the filling takes place under a hood provided with ultraviolet lamps. After each day's operation all parts of the machine which come in contact with the penicillin solution are dismantled, thoroughly cleaned, and then sterilized. To check the operation of the filling needles sample vials are removed at frequent intervals and the weight of their contents accurately determined.

The penicillin solution which has been measured into the vials is then frozen. In this operation the trays of filled vials are carried by a conveyer through a bath maintained at a low temperature. After leaving the bath the trays are loaded on hand trucks and placed in a refrigerated vault at approximately −40° C. until ready to dry.

Dry penicillin is extremely hygroscopic and will absorb moisture from the air except under conditions of very low humidity. For this reason, in the rooms in which the drying and stoppering operations are carried out, the air is not only filtered and sterilized, but is dried to a relative humidity of 10% or less.

Drying is carried out in vacuum shelf dryers of conventional type. Two vacuum systems are used, one is connected to pumps of high capacity which evacuate the dryers rapidly at the start of the operation. In the second system, which is used for the actual drying, oil diffusion pumps are used to furnish the high vacuum required, the absolute pressure at the end of the operation being only a few microns. Although penicillin holds moisture extremely tenaciously, the dry product which is obtained contains no more than 1% of moisture as a general rule.

After drying it is necessary next to stopper the finished product. Ordinarily the mere insertion of a rubber stopper in a vial would hardly be considered a very difficult technical problem. However, the extreme hygroscopicity of penicillin and the requirement that absolute sterility be maintained have complicated the operation materially.

The stoppers used, the conventional diaphragm type with certain modifications designed to facilitate cleansing, are first inspected and then agitated with a hot detergent solution. After thorough rinsing they are next agitated with a hot dilute hydrochloric acid solution. This is followed by thorough rinsing until chloride-free, the final rinses being with pyrogen-free distilled water. They are then sterilized in live steam under pressure and thoroughly dried. Representative samples are tested for moisture content, which must meet rigid specifications, and for pyrogens, which must be absent.

Stoppering is carried out in a room designed for this one purpose only. Scrupulous cleanliness and rigid sterile conditions are observed at all times.

The actual stoppering takes place under hoods in small cubicles, located around a revolving turntable. The trays of vials of dry penicillin, still covered by the lid, are placed on the turntable, together with boxes of sterilized, dry, rubber stoppers. As these pass the cubicles each operator removes a tray of penicillin vials and one of stoppers. The covers are then removed under the hoods located at each booth. Dry sterile air is fed in at the top of each hood and blown down on and away from the vials and stoppers. The stoppers are inspected and inserted and the tray of stoppered vials returned to the turntable. As they return to the starting point they are removed and the vials are fed through slits in the wall to the capping machine.

Here an aluminum seal with removable center is applied under pressure, preventing the absorption of atmospheric moisture around the stopper. Each vial of the finished product is carefully inspected as one of the operations on the packaging line.

Random samples are taken of each lot, their number being proportional to the total number of vials in the lot. These are carefully tested to assure that the material reaching pharmacists and physicians meets rigorous specifications.

The final tests include: (1) assay for potency, (2) sterility, (3) pyrogens, (4) toxicity, (5) solubility, (6) *p*H, (7) ash, (8) moisture.

The Oxford cup test is used for the assay following the same procedure as in the laboratories of the Food and Drug Administration. As a check, each sample is also assayed by the turbidimetric method. This is a rapid procedure by which a result can be obtained in about four hours. This latter method is used on control samples taken all through the process.

The procedures followed in testing for pyrogens, toxicity and sterility are those used by the Food and Drug Administration.

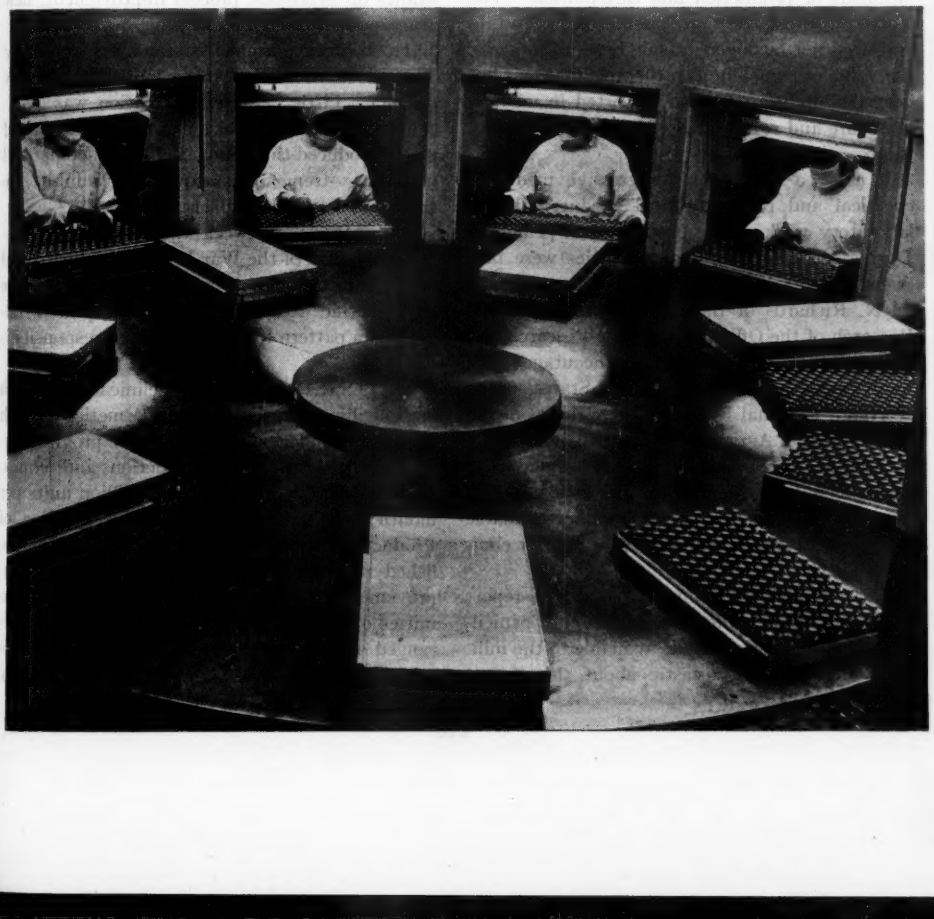
In determining solubility 10 cc. of freshly boiled and cooled distilled water, entirely free of suspended particles, are injected into the vial by means of a hypodermic syringe. The resulting solution must be clear and free of suspended particles or fibres. This solution is also used for the electrometric determination of its *p*H.

In carrying out the determination of ash, a few drops of sulfuric acid are added before ignition.

For the moisture determination the Abderhalden apparatus is used. The weighed sample is placed in the chamber surrounded by the vapor of boiling acetone and is dried to constant weight under an absolute pressure of 1 mm. or less; the loss in weight is calculated as moisture. Tests have shown that results by this method are in satisfactory agreement with those obtained by the Food and Drug Administration procedure, where the sample is dried to constant weight over phosphorous pentoxide in a vacuum desiccator at room temperature.

Despite this complex production process, the modern techniques of pharmaceutical and chemical manufacturers have not only made it possible to supply the full needs of the armed forces for penicillin but have pushed production to the point where this new drug can now be dispensed by pharmacists throughout the country.

STOPPERING the vials (below) takes place under hoods in cubicles around a revolving turntable. The hygroscopicity of dry penicillin and the need for sterile conditions complicate this operation.



PENICILLIN

PRODUCTION AND DISTRIBUTION

by FRED J. STOCK

CHIEF, DRUGS AND COSMETICS BRANCH, CHEMICALS BUREAU, WAR PRODUCTION BOARD

RELEASE OF PENICILLIN TO RETAIL PHARMACISTS CULMINATES PROGRAM WHICH HAS INCREASED PRODUCTION A THOUSAND-FOLD IN ONLY TWENTY MONTHS, HERE IS HOW IT WAS DONE

PENICILLIN is the safest, most widely effective and dramatic therapeutic agent discovered since the turn of the century. That penicillin production had to be developed during a period of wartime restrictions on manpower, materials and equipment presented unusual problems of production and distribution. The story of the discovery of penicillin has been told and need not be covered again here. Similarly, the value of penicillin as a therapeutic agent has been definitely established, reported in medical literature and reviewed elsewhere in THIS JOURNAL.

In 1941 a number of progressive medicinal chemical and pharmaceutical producers began laboratory work on the production of penicillin. During 1942 very limited quantities were produced by these firms in cooperation with Dr. A. N. Richards of the Committee on Medical Research of the Office of Scientific Research and Development. Dr. Richards encouraged the first production in the United States. The Northern Regional Research Laboratories of the Department of Agriculture did much of the early production development work and found that corn steep liquor increased the yields. Such quantities as were produced were made available to the Committee on Medical Research for clinical study.

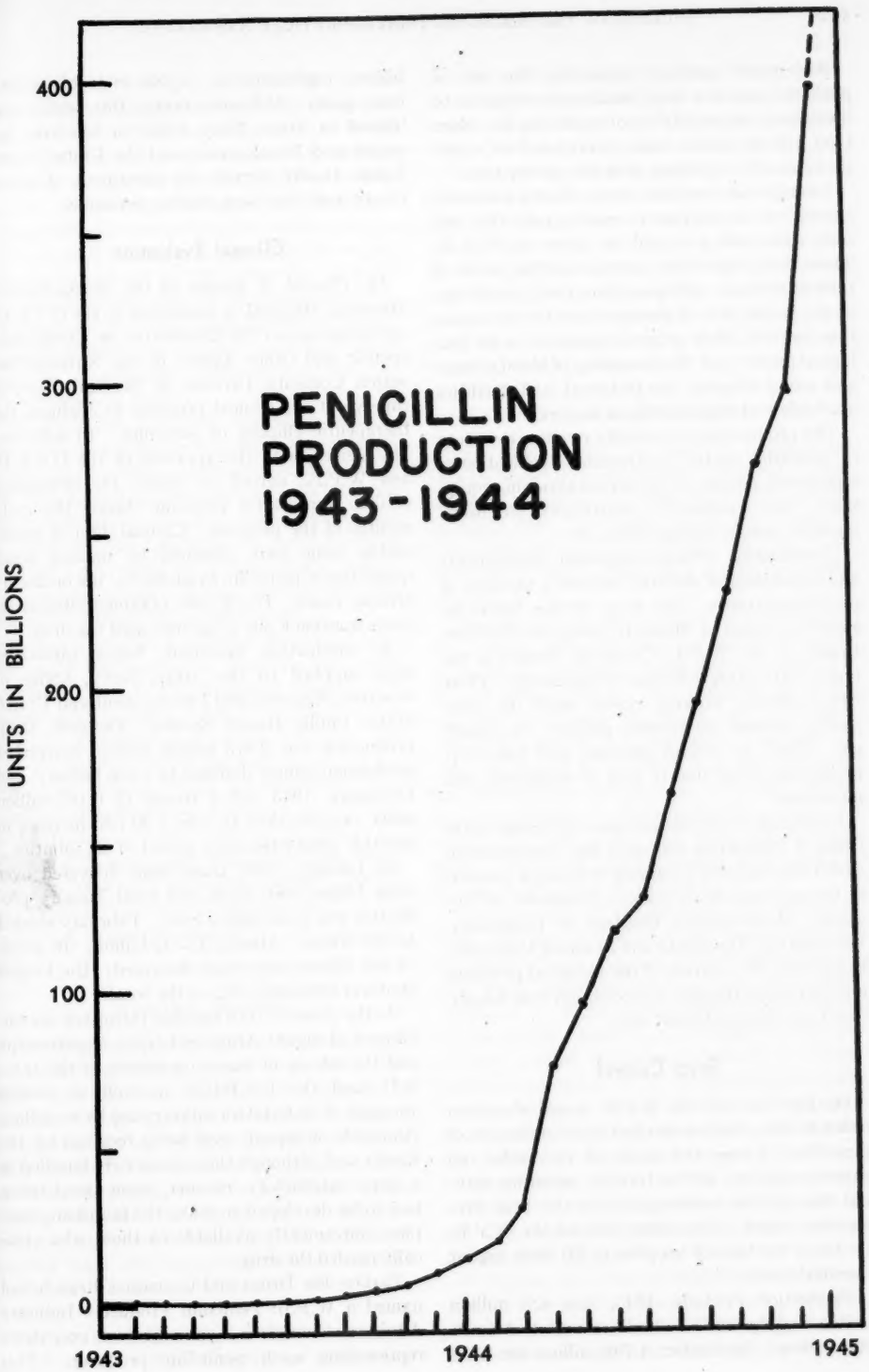
Early in 1943 the Committee began to report the results of its clinical investigation of penicillin. The results were made available to the military who were enthusiastic about this potent antibiotic agent.

In May, 1943, it became evident that this new drug would be needed by the military services in large quantities. Total production for the first five months was a mere 400 million units (4000 packages of 100,000 units). Representatives of the Drugs and Cosmetics Branch met with Dr. Richards and his associates in May, 1943, and discussed the first production program. Chemical and drug producers were eager to go ahead, but they needed War Production Board assistance for materials and equipment. The size of the production program had to be established.

The various phases of the program had to be coordinated, including priorities for equipment, technical information, further clinical evaluation, studies on synthesis, process improvement, new drug applications, size and type of package, stability, storage requirements, standards, and many other problems incident to the launching of a new product. Little definite information was available regarding the behavior of the delicate mold which produced this complex chemical compound possessing tremendous power to inhibit the growth of certain organisms.

The meetings between the Drugs and Cosmetics Branch of the War Production Board and the Committee on Medical Research of the Office of Scientific Research and Development developed a pattern for operations and responsibilities.

The O.S.R.D. continued its clinical evaluation of penicillin; the Drugs and Cosmetics Branch of W.P.B. undertook a production program including priorities for construction and equipment. A production goal of 200 billion units per month was originally set. W.P.B.'s Office of Production Research and Development established research groups to study technical improvement of penicillin production. The Committee on Medical Research of O.S.R.D. also arranged a program of chemical studies to determine the structure of penicillin with a view toward synthesis.


PENICILLIN
PRODUCTION
1943-1944
UNITS IN BILLIONS
400
300
200
100
0
1943
1944
1945

Widespread publicity regarding the use of penicillin caused a large number of companies to investigate the possibility of producing it. More than 175 companies were interviewed or corresponded with regarding penicillin production.

Twenty-one companies were selected and integrated into a program to receive priorities and such assistance as could be given by W.P.B. These companies were selected on the basis of their experience with penicillin, their knowledge of the production of chemicals by the fermentation method, their general experience with biological products or the processing of blood plasma and serum albumin, the technical staff, facilities available and other pertinent factors.

The production of penicillin requires a variety of scientific control instruments, critical steel equipment, motors, filters, air-conditioning equipment, heat exchangers, centrifuges, sterilizers, vacuum pumps, drying ovens, etc.

Coordination of these equipment requirements and scheduling of delivery became a problem of major importance. The Army Service Forces assigned a number of officers to assist the Facilities Branch of the W.P.B. Chemicals Bureau in the task of expediting deliveries of equipment. These Army officers, working closely with the companies, assured equipment delivery on schedule. Thus, by careful planning and follow-up, production delay due to lack of equipment was minimized.

Invaluable technical assistance was given by the Office of Production Research and Development, which also initiated a number of research projects at Pennsylvania State College, University of Wisconsin, Massachusetts Institute of Technology, University of Minnesota and Stanford University to assist in the solution of the technical problems incident to the transfer of production from laboratory to commercial plant scale.

Strict Control

On July 16, 1943 the W.P.B. issued allocation order M-338, which controlled the distribution of penicillin. Under the terms of this order, no person could use, sell or transfer penicillin without the specific authorization of the War Production Board. This order enabled the W.P.B. to direct the limited supplies to fill most urgent essential needs.

Production in June, 1943, was 425 million units; in July, 1943, 762 million units; August, 906 million; September, 1,788 million (or 1.788 billion, equivalent to 17,880 vials of 100,000 units each). Deliveries during this period were limited to Army, Navy, Office of Scientific Research and Development and the United States Public Health Service for treatment of Coast Guard and Merchant Marine personnel.

Clinical Evaluation

Dr. Chester S. Keefer of the Massachusetts Memorial Hospital, a consultant to the O.S.R.D. and chairman of the Committee on Chemotherapeutic and Other Agents of the National Research Council's Division of Medical Sciences supervised the clinical program to evaluate the therapeutic efficacy of penicillin. In addition, Dr. Keefer, with the approval of the O.S.R.D. and W.P.B., agreed to handle the emergency civilian appeals for penicillin during the early months of the program. Clinical data of inestimable value were obtained by making small quantities of penicillin available to "life or death" civilian cases. Dr. Keefer obtained clinical reports from each physician who used the drug.

As production increased, larger quantities were supplied to the Army, Navy, Office of Scientific Research and Development and United States Public Health Service. October, 1943, production was 2.873 billion units; November production almost doubled to 4.846 billion; and December, 1943, set a record of 9.195 billion units, or equivalent to over a 20-fold increase in monthly production in a period of six months.

In January, 1944, three firms delivered over three billion units each, and total January production was 12.55 billion units. February totaled 18.726 billion; March, 32.192 billion. In April, 74.964 billion units were delivered; the largest producer delivered 50% of the total.

At the close of April supplies permitted the fulfillment of urgent Army and Navy requirements and the release of larger quantities to the O.S.R.D. and the U.S.P.H.S. as well as limited amounts of material for military use by our allies. Hundreds of appeals were being received by Dr. Keefer and, although these cases were handled in a very satisfactory manner, some mechanism had to be developed to make the increasing supplies conveniently available to those who critically needed the drug.

Earlier, the Drugs and Cosmetics Branch had named a W.P.B. Penicillin Producers Industry Advisory Committee, composed of executives representing each penicillin producer. This

Committee met frequently with W.P.B. to discuss problems of production, raw materials, equipment and additional subjects. The Penicillin Producers Committee was asked to develop a program for limited distribution of penicillin. Supplies were wholly inadequate to permit normal distribution through regular trade channels. Demands were in excess of the available supply although full use of the drug had not been developed because some of the clinical studies were not complete. Supplies, however, were sufficient to permit more to be released to civilians than could be conveniently approved through Dr. Keefer's office in Boston.

The Penicillin Producers Industry Advisory Committee developed an excellent plan to permit limited distribution to a selected list of hospitals. The plan submitted was approved by all interested governmental agencies. This plan proposed to distribute such quantities as could be made available for civilian use after military and research requirements had been met by establishment of a central office to clear all orders.

Chicago was selected as the location of this office because of its geographic location and space available in the W.P.B. Regional Office. This central office became known as the Civilian Penicillin Distribution Unit (C.P.D.U.). Dr. John N. McDonnell was appointed chief of the Unit. An Advisory Panel was established composed of representatives of the United States Public Health Service, Office of Scientific Research and Development, War Production Board, W.P.B.'s Office of Civilian Requirements, American Medical Association and the chief of the Drugs and Cosmetics Branch, W.P.B., as chairman.

Hospitals Selected

The Panel selected 1000 hospitals to receive the initial distribution of penicillin. The hospitals were selected primarily on the basis of the number of beds, number of admissions, type of institution, and geographic location. Each of these hospitals was assigned a monthly quota based on these factors. The hospitals became "depot hospitals" for the proper storage and further distribution of penicillin. In addition to meeting their own requirements they were to supply the requirements of other hospitals in their area.

The depot hospitals placed their orders with the Civilian Penicillin Distribution Unit which checked and charged them against their quotas. These orders were forwarded the same day to manufacturers who had penicillin available. Often the hospital orders were phoned by C.P.D.U. to the manufacturer, thus permitting shipment on the day the order was received by C.P.D.U. Manufacturers invoiced the hospitals in accordance with their usual policy. All hospitals were advised of the plan by W.P.B. and were supplied with copies of Dr. Keefer's report on the use, indications, contraindications, and mode of administration of penicillin (see THIS JOURNAL, 5: 166, 1944).

Expanding Distribution

Since May 1, 1944, the monthly quotas have been increased by approximately 40% and the list of depot hospitals now includes 2700 hospitals. In May, 1944, 12.2 billion units were released through the C.P.D.U. for civilian use. In June, 14.3 billion, and in July, 15.4 billion units were released.

Normally we, as pharmacists, do not consider the problem of distribution. Supplies are made available by placing an order and deliveries are prompt and complete. This condition, however, can only exist when supplies are normal, when adequate amounts can be made available by basic producers to permit a free and complete flow through the distributional "pipe line" to the retail pharmacist. Interrupt that flow or curtail it at the source, and chaos will result—panic, overbuying, unfilled orders with resultant lack of supplies for critical needs.

It was estimated at that time that 40 billion units (400,000 packages) would be required to fill the distribution pipe line so that retail pharmacists could order penicillin as they order other medicinal products. Forty billion units could not be spared. There was no assurance that increased military requirements would not necessitate a curtailment of the limited supplies available for civilians. Forty billion units was the approximate total consumption of penicillin by the hospitals during the first three months (May–July) operation of C.P.D.U. Certainly the use of penicillin for urgently needed cases would have had to be delayed if the normal distributional methods had been used.

If normal channels of distribution were to take the responsibility for distribution, then the government had an obligation to guarantee to make adequate amounts available at intervals.

Since the government, in view of military requirements, could not give such assurance, an alternate plan, the C.P.D.U. plan, to make penicillin available until supplies became adequate was necessary and in the best national interest. However, it was realized that this control by C.P.D.U., established by the W.P.B., must be removed at the earliest possible date.

The production curve turned sharply upward in May, 1944, to 94.132 billion units and to 117.527 billion in June, 1944, an increase in monthly production of over 250 times attained in one year. By January, 1945, production had risen to over 394 billion units (3,940,000 packages) and was rapidly headed for 450 billion units per month.

Millions upon millions of packages of penicillin are produced each month by the penicillin laboratories. All military requirements of our country and our allies are now satisfied. Supplies are ample to initiate distribution through customary professional channels.

The Penicillin Producers Industry Advisory Committee met on January 30, 1945, to consider a plan to transfer sales from the controlled procedure of the C.P.D.U. to normal commercial methods. The plan which was developed received the approval of the various governmental organizations and interested groups.

The plan permitted normal distribution of penicillin on and after March 15. The 2700 hospitals that had been serving as depot hospitals used 350,000 packages (35 billion units) in January; consumption during February by these hospitals was 380,000 packages (38 billion units). The W.P.B. released 1,280,000 packages (128 billion units) for distribution through commercial channels. Present plans call for release of approximately 1,500,000 packages in April and similar quantities each subsequent month. This material is made available in packages of 100,000 units of sodium penicillin for human parenteral medication.

The material should be stored at 2° to 10° C. Each package is dated showing the effective life of the product. All commercial manufacturers have had sufficient experience to permit the Food and Drug Administration to allow a year's dating on the product.

Penicillin is not in sufficient supply to permit its use in manufactured pharmaceutical products, such as pills, lozenges, ointments and other dosage forms. Marketing of these pharmaceuticals will have to await greater availability of the drug, further clinical research and product development.

Industry has done an outstanding job in the production of this vital medicine. The splendid cooperation of the management and the employees of the producing companies merits commendation.

In twenty months production has increased a thousand-fold from 4000 packages per month to over 4,000,000 packages per month (a sufficient supply for all present parenteral use). In July, 1943, the price was $20 per package of 100,000 units. Prices have declined to about $2 per package and further price reductions are anticipated. In 1944, a total of 16,633,000 packages (1663 billion units) were produced with a total value of approximately $35,000,000. In 1945, the production of units of penicillin will increase three or four times over 1944 and will have an approximate value of $75,000,000.

Penicillin takes its place among the valuable drugs available to the physician from his pharmacist. Penicillin, born during the war emergency, matured rapidly to relieve the infections and sufferings of battle, will serve mankind in years to come. Penicillin is the first of an entirely new group of therapeutic agents—the antibiotics.

BELLADONNA USE IN MUMPS NOT SUPPORTED BY TESTS

Reports that belladonna may shorten the course of mumps and reduce the incidence of complications are not supported by a study of 124 cases among soldiers by Lt. Col. H. W. Potter, MC, ORC, and Capt. L. H. Bronstein, MC, AUS (*Ann. Int. Med.*, 21: 469, 1944).

The 42 patients who received belladonna had as much discomfort as the 82 untreated patients, and there was no significant difference in the duration of illness, the incidence of complications or the duration of orchitis.

Treatment in the tests consisted of $^1/_{100}$ gr. atropine sulfate, followed by 1 cc. Tincture of Belladonna by mouth every two hours until atropinization was achieved. Atropinization was maintained by administration of the Tincture until the patient recovered or gastro-intestinal discomfort necessitated withdrawal.

PENICILLIN

CONSIDERATIONS IN THERAPY

by JOHN S. LOCKWOOD*

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AUTHORITATIVE REVIEW OF THE CURRENT STATUS OF PENICILLIN TREATMENT AND ITS LIMITATIONS

THE development of penicillin as a practical chemotherapeutic agent is an outstanding chapter in the medical history of the present war. This potent antibacterial product of the mold, *Penicillium notatum*, was originally discovered by Sir Alexander Fleming, in 1929.[1] However, its possibilities as a chemotherapeutic agent were not demonstrated until ten years later.

Moved by a pressing need for a drug which would be superior to the sulfonamides in controlling staphylococcic and other infections of war wounds, Sir Howard Florey and his colleagues at Oxford reopened the study of penicillin in 1939. Two years later they were able to report such impressive success in the treatment of severe staphylococcic infections[2] that work on penicillin was taken up energetically in the United States and pursued as an urgent part of our war effort. Several large pharmaceutical concerns supplied facilities and personnel for study and production of the drug, and wholehearted support was afforded by the U. S. Department of Agriculture and the War Production Board.

A large-scale clinical trial was organized and conducted by the Committee on Medical Research of the Office of Scientific Research and Development in collaboration with the National Research Council, a study in which many physicians and laboratory scientists participated.[3]

Evidence of the success of this joint enterprise is to be found in the fact that total production of penicillin has increased fifteen-thousand-fold since 1942, the practical considerations governing use of penicillin are defined in all major respects, and the drug is being widely used in the treatment of hundreds of thousands of patients in military and civilian hospitals all over the world. Never before has a new discovery in the pharmaceutical field moved so rapidly and on such a large scale from the laboratory to the bedside.

Indications

(See 4a, b, c, d, e, f; selected general articles)

Penicillin is *indicated* in the treatment of infections due to bacteria against which this drug is known to be active and in which the infection is of sufficient severity to warrant subjecting the patient to the discomfort and inconvenience attending its administration. The infections in which penicillin is of notable usefulness may be grouped in accordance with the following bacteriologic classification:

1. *Staphylococcus Infections*—including bacteremia, cellulitis, carbuncle, pneumonia, empyema, meningitis, osteomyelitis and suppurative arthritis. In these infections penicillin is clearly superior to the sulfonamides.

2. *Pneumococcus Infections*—including pneumonia, endocarditis, empyema, suppurative, arthritis and meningitis. In these infections penicillin is not notably superior to sulfadiazine and other sulfonamides except in empyema and meningitis where local treatment with penicillin is more efficacious than is local sulfonamide treatment. Penicillin is especially useful in those cases of pneumococcic infections in which sulfonamide treatment has proved to be inadequate.

3. *Meningococcic Infections*—including meningococcemia and meningitis. Results with penicillin in these infections have not been any better than those with sulfonamides, but penicillin is indicated in cases resistant to sulfonamide therapy.

4. *Gonococcic Infections*—including urethritis, salpingitis and arthritis. Penicillin will bring

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about prompt cure in a higher proportion of cases than will any of the sulfonamides and will usually cure those cases which have failed to respond to sulfonamide treatment.

5. *Gas Gangrene Infections.*—Penicillin is the best antibacterial agent for prevention and treatment of infection with the organisms causing gas gangrene in severe wounds. However, penicillin will not prevent or cure infection in cases where surgical treatment has been inadequate, and should be used only as an adjunct to surgery, to prevent spread of the infection into previously healthy tissues.

6. *Streptococcus Viridans Infections.*—Penicillin is clearly indicated in the treatment of subacute bacterial endocarditis due to this organism, and the results are far superior to those obtained with any other chemotherapeutic agents. However, it will not be effective in those cases in which a particular strain of the organism is insensitive to the action of the drug, as shown by laboratory tests. Prolonged treatment with large doses is required.

7. *Hemolytic Streptococcic Infections*—including bacteremia, cellulitis, pneumonia, empyema, meningitis, osteomyelitis and suppurative arthritis. Penicillin is especially indicated in cases where sulfonamide treatment is unsuccessful, but in the majority of instances adequate sulfonamide treatment will be sufficient.

8. *Anthrax.*[5]—The less severe forms of this infection have been shown to be remarkably susceptible to penicillin. It remains to be shown that the drug will be equally successful in the severe cases, but better results are to be expected with penicillin than with any other method of treatment.

9. *Syphilis.*[6]—Final definition of the usefulness of penicillin in syphilis must await the outcome of extensive controlled studies now in progress. However, it seems probable that penicillin will occupy a leading position in the treatment of early syphilis, although best results may prove to occur when penicillin is used combined with arsenical therapy. The results with penicillin in congenital and late syphilis are less certain, but definite benefit is afforded in a certain proportion of cases. Adequate evaluation of the treatment of syphilis with chemotherapeutic agents is exceedingly difficult and patients with this disease should not be treated with penicillin by physicians who are not thoroughly informed of the complexities and pitfalls to be encountered.

Penicillin is *not indicated* in the treatment of intestinal infections due to members of the colon-typhoid-dysentery group. Very few strains of gram-negative bacilli are susceptible to this drug and penicillin is valueless in infections due to Brucella, *Hemophilus influenzae* and *Pseudomonas pyocyanea.* No therapeutic benefit is to be expected in cholera, plague and tuberculosis. Penicillin has been shown to be valueless in ulcerative colitis, rheumatic fever and rheumatoid arthritis. Penicillin is not indicated in the treatment of cancer and leukemia unless there happens to be a coexisting infection with a penicillin-sensitive organism.

Methods of Administration

Oral Administration.—A large proportion of penicillin given by mouth is inactivated by the acid in the stomach, and of that which goes on into the small intestine, only a limited proportion is absorbed. Therefore, penicillin in any form now available is not efficiently utilized when administered by mouth. No special vehicle or capsule for oral administration has yet been devised which will permit efficient treatment through the oral route.

Under the most favorable conditions oral administration will require the use of three or four times as much of the drug, and even then the therapeutic response is likely to be somewhat erratic. Consequently, oral administration of penicillin is not yet recommended, on the basis both of efficiency and of cost.

Parenteral Administration.—Solutions of penicillin may be given by intravenous or intramuscular routes. If the intravenous route is employed the total twenty-four hour dosage is dissolved in an appropriate amount of a solution of isotonic saline or 5% glucose and given by continuous drip at a uniform rate throughout the twenty-four-hour period. Alternatively, the drug may be given by the intramuscular route. This may be done by a similar continuous drip procedure using a small needle and an amount of solution not exceeding 300 cc. per day, or by intermittent injection.

If intermittent intramuscular injection is employed the most efficient procedure requires that the twenty-four-hour dose be divided into twelve fractions given at two-hourly intervals. Under no circumstances should the interval exceed three hours because most of the drug injected will have been excreted by the end of the second

hour, and thereafter very little of the drug will be in circulation until the next dose has been administered.[7]

Special Vehicles for Delaying Absorption.—Considerable effort is being expended on the development of vehicles which will hold penicillin at the site of injection and liberate it slowly over a period of many hours. Successful solution of this problem would obviate the necessity for multiple injections of the drug and would result in maintenance of higher concentrations of penicillin in the circulating body fluids. Such a vehicle must not inactivate the drug and must be reasonably well tolerated by the body tissues and must not of itself be toxic.

A vehicle consisting of anhydrous beeswax and peanut oil has shown promising possibilities and is now being carefully investigated. Using this vehicle Romansky[8] has successfully treated gonorrhea with a single injection of 150,000 to 300,000 units of penicillin, resulting in detectable concentrations of penicillin in the blood for twelve hours or more. (Gonorrhea is one of the diseases most susceptible to penicillin and it may not be assumed that similar success can be obtained with this method in all types of infections in which penicillin is indicated.)

Topical Administration.—Penicillin does not pass readily from the circulating body fluids into closed cavities such as the pleura, pericardium, joints, and subarachnoid space. In infections of these regions it is therefore preferable to supplement systemic treatment with introduction of the drug locally into the affected cavity.[7] Local treatment is indicated, for the same reasons, in carefully selected cases of localized soft tissue abscesses.

When penicillin is administered topically in such cases the drug is dissolved in physiological saline in a concentration of 500 to 5000 units per cubic centimeter and the amount introduced will depend upon the size of the cavity and the amount of fluid which can conveniently be withdrawn prior to its injection.

In meningitis the intraspinal injection of 10,000 to 20,000 units every one or two days will provide an adequate supplement to systemic treatment. Similar or larger doses may be employed locally in empyema. In localized abscesses and other surgical infections an unwarranted delay in carrying out surgical drainage may be dangerous to the patient and, as a rule, local penicillin treatment should be used in conjunction with surgical drainage rather than as an attempt to replace surgery.

Dosage

The principles of dosage in penicillin therapy have been to a large extent related to the status of supply. Since penicillin is virtually without toxicity the upper ceiling in dosage is directed toward the point of "diminishing returns." If cost and supply do not impose any restrictions (at the date of this writing they are still very real) doses of 400,000 to 500,000 units per day will be satisfactorily tolerated and may be indicated in selected cases of very severe or demonstrably resistant types of infections. However, in most of the acute infections in which penicillin is indicated a total twenty-four-hour dose of 150,000 to 250,000 units need not be exceeded. If no therapeutic response is obtained with the latter amount it is unlikely that still larger doses will be of significant value.

The method of administration, with particular regard to maintenance of a continuously adequate and measurable concentration of penicillin in the blood, is of greater importance than the employment of extremely high total dosage. The duration of treatment will vary greatly according to the disease being treated, but in most acute infections other than gonorrhea continuation of treatment for at least a week will probably be necessary. Failure to obtain any detectable clinical response from adequate doses given for a week rather definitely indicates that the infection is resistant to penicillin and that further administration of the drug is probably futile. If the disease responds at all, an improvement in the patient's condition will usually be recognized within three days. The drug should be continued for several days after subsidence of clinical signs of infection in order to avoid the occurrence of a relapse.

In *chronic* infections, particularly those of the bone, heart valves and lungs, it is usually necessary to continue the administration of penicillin for three or four weeks. In *chronic* infections of bone and lungs the optimum time for carrying out the associated surgical procedures which are usually called for will be after the lesion has shown its maximal response to the course of drug treatment.[4, 6, 7]

The use of penicillin is contraindicated in diseases which cannot be classified as specific infections due to penicillin-susceptible bacteria, be-

cause it is valueless except under these conditions. Penicillin is a remarkable drug when used in accordance with rational principles. However, the fact that it is nontoxic cannot justify its indiscriminate administration in diseases where it could not possibly be of therapeutic benefit.

In the case of most drugs there exists a sufficient measure of toxic potentiality to discourage therapeutic trials which are not backed by adequate justification through careful clinical and laboratory study. However, in the case of penicillin the principal restraints against wasteful and indiscriminate treatment will be the cost of the drug, the present necessity for administering it parenterally, and finally, the honesty and intelligence of physicians and pharmacists.

As yet, no true incompatabilities are recognized which would prevent penicillin being safely used in conjunction with other forms of treatment. The use of penicillin would be contraindicated in individuals who have demonstrable sensitivity to the drug. (See below.)

Limitations of Penicillin

Obviously penicillin can be of therapeutic value only in infections due to penicillin-susceptible bacteria. However, the drug must also be transported to the site of the infection in adequate concentrations. As previously mentioned, penicillin administered by systemic routes is not transported in satisfactory concentration to the fluid contents of infected body cavities, and therefore in such cases this limitation must be overcome by topical administration of the drug.

Penicillin does not directly destroy bacteria in the body. It tends rather to cause cessation of bacterial reproduction and thus undermines the ability of the bacteria to resist destruction by the natural immune mechanisms of the host. It is the combined action of penicillin with the host's immune response which leads to recovery from the infections. When devitalized tissue is present, as in an abscess or an area of gangrene, neither penicillin nor mechanisms of immunity will succeed in controlling the growth of bacteria in the devitalized zone. Proper treatment of the patient under these conditions calls for a resort to surgical removal of the devitalized tissue. Once this has been achieved penicillin will aid in preventing any spread of the infection into previously normal and uninvolved zones.

Another limitation of penicillin is imposed through the necessity for administering it parenterally. This may be a helpful limitation to the extent that it discourages the use of penicillin in the treatment of all sorts of minor infections, including the common cold, chronic sinusitis, etc. As long as the amount of penicillin available for civilians is subject to limitations this factor undoubtedly helps to save the available supply for treatment of the more serious disorders in which it has a definite life-saving value.

Still another limitation of penicillin is related to its chemical instability. The dry powder will retain its activity for many months at refrigerator temperature provided it can be kept in an anhydrous state. Once penicillin comes in contact with moisture it begins to deteriorate and the rate of deterioration increases with temperature. The activity of penicillin in solution can be destroyed in a few minutes by boiling, and a considerable proportion of the activity is lost when a solution is allowed to stand at room temperature for as little as twenty-four hours; therefore, the need for careful handling of penicillin preparations is obvious. Furthermore, penicillin will not retain its activity for long when it is mixed with aqueous ointment bases or other vehicles which contain water.

Solutions of penicillin are bacteriostatic only for the susceptible species of bacteria. Air borne bacteria of many varieties will easily multiply in a solution of penicillin; therefore great care must be exercised to prevent the entrance of contaminating bacteria into vials and flasks containing dissolved penicillin.

Untoward Reactions

Penicillin is remarkably free from any tendency to produce serious untoward reactions. Being rather alkaline, it is moderately irritating to the tissues when applied in concentrated solution. Patients may, therefore, complain of transient burning pain at the site of penicillin injection. This is not serious but becomes decidedly troublesome to patients when the injections must be repeated at two-hour intervals, day and night.

Solutions of penicillin given intravenously may produce irritation of the lining of the vein with resulting thrombosis and local pain and tenderness. It is rarely possible to continue injection of penicillin into any single vein for more than forty-eight hours. Because of this irritant property, concentrated solutions of more than 1000 units per cubic centimeter should not be used on delicate membranes such as the conjunc-

tiva, although the muscle will tolerate injections of aqueous solutions in concentrations as high as 10,000 units per cubic centimeter.

Apart from these local reactions to penicillin, the only important toxic phenomena are concerned with allergic or sensitivity reactions to penicillin or to the impurities which are associated with it. These reactions may be manifested by urticaria or by development of a maculopapular rash.

A small percentage of patients will tend to develop fever after several days of penicillin treatment and it may at times be difficult to differentiate this "drug fever" from a fever which might occur from the infection itself. It is not yet known how long this state of reactivity can persist in an individual and it may become a problem of increasing importance in therapeutics in the future.

Fortunately, these reactions rarely develop until after the acute phase of infection has subsided, and therefore it is only rarely necessary to withhold penicillin from patients who are in serious need of continued treatment.

Penicillin Resistance

Many varieties of bacteria are inherently resistant to the bacteriostatic action of penicillin. The largest group of such bacteria are the gram-negative bacilli which are inhabitants of the intestinal tract in health and disease. Many of these types of bacteria contain or produce an enzyme which rapidly destroys penicillin. This enzyme is called "penicillinase" and its presence accounts in part for the innate resistance to penicillin of the related species.

However, other organisms may acquire a resistance to penicillin through prolonged contact with low concentrations of the drug. In the course of treatment of certain types of infections one may find that the organisms will develop an increasing tolerance to the drug. This can be of particular importance in chronic infections where prolonged treatment is required. This presents an important reason for starting treatment with doses capable of producing a rapid chemotherapeutic response. For example, unsuccessful therapeutic trial of oral penicillin might serve to increase the resistance to penicillin of the infecting organisms and thus render subsequent parenteral therapy less effective.

Summary

1. Penicillin treatment is indicated only in those infections in which the causative microbe is susceptible to its antibacterial action.

2. The superiority of penicillin over the sulfonamides is most clearly demonstrable in severe infections due to the staphylococcus, in gonorrhea, and in subacute bacterial endocarditis.

3. Penicillin is preferably administered parenterally, by continuous intravenous or intramuscular drip or by intermittent intramuscular injection at two-hour intervals.

4. In certain types of infections of closed body cavities it is desirable to employ direct local injections of solutions of the drug.

5. Penicillin should be used only as an adjunct to surgery in infections characterized by sloughing and gangrene.

6. Penicillin is remarkably free from toxic actions, although occasional skin rashes and drug fevers are seen.

7. When employed in accordance with sound principles, penicillin is a drug of remarkable power and usefulness. It should not be wasted in treating diseases in which it could not be of specific value.

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PENICILLIN

PHARMACY AND FORMULATION

by R. J. DAHL

E. R. SQUIBB AND SONS

PENICILLIN COMPOUNDING DEMANDS UNDERSTANDING OF PROPERTIES AND ASEPTIC TECHNIQUE; LATEST DATA ON PHARMACEUTICAL FORMS GIVEN

IT IS with no little temerity that one approaches the task of attempting at this relatively early date to delineate properties of penicillin which will aid in the intelligent compounding of penicillin pharmaceutical forms. Those who have occupied themselves with the study of the chemical and physical properties of penicillin will give warm support to that temerity. For the eager clinician has far outstripped his laboratory colleague in his application of the drug in forms for which the laboratory investigator can offer but scant data regarding their properties or regarding means to assure optimal retention of essential properties. Even many of the known chemical properties of penicillin must, for security reasons, still be withheld.

Nonetheless, it must be recognized that clinical studies have demonstrated that penicillin in a variety of pharmaceutical forms has proved effective, however inadequate in some respects those forms may be; that pharmacists will be called upon by physicians to compound pharmaceutical forms; and that therefore information on compounding, although tentative, will be of some use. Doubtless more comprehensive reports will appear later as greater knowledge of the properties of penicillin and lifting of security restrictions permit.

Of greater importance to the pharmacist than the origin and manufacture of penicillin is an understanding of its nature and of its chemical properties.

There is definite evidence of at least three "penicillins," designated, respectively, as G, F and X (or allopenicillin), which differ slightly in chemical structure. Penicillin G, F and X differ also in their degree of antibiotic effect upon certain organisms. Although the evidence is somewhat fragmentary since studies are not complete, it appears that *in vitro* penicillin F has less effect upon *B. subtilis* but more effect upon *Staphylococcus aureus* than does penicillin G. Penicillin X has a greater effect *in vitro* upon *Klebsiella pneumoniae*, type A, and clinically upon *Neisseria gonorrhoeae* than either penicillin G or F.

In addition to the sodium salt of penicillin, the calcium, strontium, barium, ammonium, lithium and magnesium salts have been prepared and subjected to limited laboratory test and some also to limited clinical test. Calcium penicillin has had clinical employment, particularly by one branch of the military services.

Penicillin G was first crystallized as the sodium salt in July, 1943, by MacPhillamy and Wintersteiner of the Division of Organic Chemistry of the Squibb Institute for Medical Research (*cf.* Coghill, R. D., *Chem. Eng. News*, 22:588, 1944). This finding, together with complete evidence of analytical composition, was submitted to the Committee on Medical Research of the Office of Scientific Research and Development at that time.

Free penicillin is a strong dibasic acid and readily forms salts with bases. It has also been possible to esterify the carboxyl group. The commercial penicillin sodium of today is not a chemical entity, being composed of approximately 60% penicillin sodium, the balance being a mixture of the sodium salts of most of the other organic acids contained in the extract.

It is readily soluble in water, ether, acetone, glycerin, propylene glycol and alcohol, but the latter solvent quickly destroys its activity. It is less soluble in benzene, chloroform and carbon tetrachloride. The standard solution for injection purposes—100,000 Oxford units in 20 cc. of Sterile Isotonic Solution of Sodium Chloride—has a *p*H range of 6.3 to 6.8. At *p*H values below

3 and above 9, penicillin sodium rapidly loses activity.

Penicillin sodium is rapidly destroyed by acids, alkalis, oxidizing agents, certain heavy metals and enzymes produced by many bacteria. In its pure crystalline form it is relatively stable, but at temperatures above 10° C. in the presence of moisture a gradual loss of potency occurs. Because of its labile characteristics, penicillin sodium should always be kept at a temperature below 10° C., irrespective of what form it is in.

Stability is not only affected by temperature, however, for moisture also adversely affects the activity of penicillin sodium. The salt is very hygroscopic and all possible protection against exposure to moisture should be provided. A solution of penicillin sodium at *p*H 6.3 to 6.8 will retain its potency without significant loss for one week to a month at 10° C. or below, but it is considered inadvisable to hold such solutions for more than one to two weeks since the rate of deterioration may be accelerated by slight impurities in the diluent. At room temperature, fairly rapid deterioration in potency may occur after two to three days. Satisfactory retention of potency at room temperature may be expected for at least one day.

The calcium salt of penicillin G is much less hygroscopic than the corresponding sodium salt and is more stable. Light has no adverse influence on the potency of either penicillin sodium or penicillin calcium.

Absorption and Excretion

Intravenous injection of penicillin sodium produces a high initial concentration in the blood plasma—a maximum of 10 units per cubic centimeter—followed by an abrupt fall.[1] The plasma concentration peak is reached immediately after intravenous injection, decreasing 75% within fifteen minutes and 90% at thirty minutes. Only traces can be detected after three to four hours. Absorption and excretion are also rapid after intramuscular injection but slow after subcutaneous injection. A more recent report[2] has shown that after subcutaneous injection of penicillin the blood curves have been quite similar to those following intramuscular injection. As much as 100,000 units injected subcutaneously produced little discomfort. The plasma concentration peak is reached within fifteen to thirty minutes after intramuscular injection, remaining at that level for about thirty additional minutes and then declining, with only traces detectable after three to four hours.

Aqueous solutions of penicillin sodium administered orally are poorly absorbed due to inactivation by the gastric juice.[3] It is the hydrochloric acid of the gastric juice and not pepsin which is responsible for this inactivation.

That penicillin sodium can be absorbed across certain areas of the intestinal wall has been demonstrated by duodenal intubation. This method gave plasma concentration levels paralleling those following intramuscular injection.[1] By rectal instillation, the drug is poorly absorbed, being inactivated by feces and the enzyme penicillinase, produced by *E. coli*.

Injection into body cavities and tissue spaces is followed by slow absorption, appreciable amounts remaining therein many hours after injection. Intrathecal injection is also followed by slow absorption.

Diffusion studies following intravenous injection have shown that penicillin sodium does not penetrate the erythrocytes in significant amounts, a concentration less than 10% of the concentration in the plasma being attained.[1] Penicillin sodium also diffuses poorly into the spinal fluid and from the spinal fluid into blood. It does not penetrate the saliva or tears following intravenous injection. Higher concentrations than in plasma are found in bile, suggesting that penicillin sodium is at least partly excreted by the liver.

Penicillin sodium is rapidly excreted in the urine, 40 to 60% being excreted in twelve hours' time, with the greatest amount excreted within the first hour after intravenous administration. Renal impairment obviously delays the excretion of penicillin sodium and results in higher and more sustained plasma levels.

Because of its rapid excretion and the necessity for maintaining an adequate blood plasma concentration level, intravenous or intramuscular injection every three to four hours or continuous infusion is necessary. Although published figures vary widely (an indication that there is no accurate method for determining the concentration in body fluids), it is generally agreed that a concentration of 0.03 to 0.06 unit per cubic centimeter of blood constitutes a therapeutic level.

Dosage[4]

"The dosage of penicillin will vary from one patient to another depending on the type and severity of infection. In our experience recovery has followed in many serious infections following 40,000

to 50,000 Oxford units a day, in others 100,000 to 120,000 or even more is necessary. The objective in every case is to bring the infection under control as quickly as possible. The following recommendations are made at the present time with a full realization that revisions may be necessary as experience accumulates.

"It is well to remember that penicillin is excreted rapidly in the urine so that following a single injection it is often impossible to detect it in the blood for a period longer than two to four hours. It is well, therefore, to use repeated intramuscular or intravenous injections every three or four hours, or to administer it as a continuous infusion.

"A. In serious infections with or without bacteremia an initial dose of 15,000 or 20,000 Oxford units with continuing dosage as:

"1. Constant intravenous injections of normal saline solution containing penicillin so that 2000 to 5000 Oxford units are delivered every hour, making a total of 48,000 to 120,000 units in a 24-hour period. One-half the total daily dose may be dissolved in a liter of normal saline solution and allowed to drip at the rate of 30 to 40 drops per minute.

"2. If continuous intravenous drip is undesirable, then 10,000 to 20,000 units may be injected intramuscularly every three or four hours.

"3. After the temperature has returned to normal the penicillin may be stopped and the course of the disease followed carefully.

"B. In chronically infected compound injuries, osteomyelitis, etc., the dosage schedule should be 5000 units every two hours or 10,000 units every four hours parenterally with local treatment as indicated. This dosage schedule may have to be increased, depending upon the seriousness of the infection, and response to treatment. The best results are obtained in these cases when penicillin is combined with adequate surgical treatment.

"C. Sulfonamide-resistant gonorrhea: Twenty-thousand units every three hours intramuscularly for 5 doses. The minimum dosage has not been worked out completely. The results of treatment should be controlled by culture of exudate.

"D. Empyema: Penicillin in normal physiological saline solution should be injected directly into the empyema cavi y after aspiration of pus or fluid. This should be done once or twice daily, using 30,000 or 40,000 units depending upon the size of the cavity, type of infection and number of organisms. Penicillin solutions should not be used for irrigation. It requires at least six to eight hours for a maximum effect of penicillin.

"E. Meningitis: Penicillin does not penetrate the subarachnoid space in appreciable amounts, so that it is necessary to inject penicillin into the subarachnoid space or intracisternally in order to produce the desired effect. Ten thousand units diluted in physiological saline solution in a concentration of 1000 units per cubic centimeter should be injected once or twice daily, depending upon the clinical course and the presence of organisms.

"F. Bacterial Endocarditis: Penicillin is the best agent available for the treatment of bacterial endocarditis. Penicillin alone is as effective as penicillin combined with heparin. The treatment should be continued for three weeks or longer depending upon the individual case. The intramuscular route is the one of choice, although in a few instances it may be desirable to use the continuous intravenous route in order to obtain the maximum effect. The dosage should range from 200,000 to 300,000 units a day if the best results are to be obtained."

Pharmaceutical Formulation

1. Injection Forms

A. Parenteral Solutions:

The most commonly employed mode of administration of penicillin sodium has been intravenous or intramuscular injection of solutions. The following directions for preparing solutions for intravenous or intramuscular injection are contained in a report[4] by Dr. Chester S. Keefer, chairman of the Committee on Chemotherapeutic and Other Agents of the National Research Council and consultant to the Office of Scientific Research and Development:

"Penicillin is supplied in ampuls and vials of 100,000 units each. Inasmuch as penicillin is extremely soluble, it may be dissolved in small amounts of sterile, distilled, pyrogen-free water, or in sterile, normal saline solution. When large unit sizes are being used in hospitals, the contents of the ampul should be dissolved in water or saline so that the final concentration is 5000 units per cubic centimeter. This solution should be stored under aseptic precautions in the ice box, and made up freshly every day. Solutions for local or parenteral use may be diluted further, depending upon the concentration desired.

"a. For intravenous injection

"1. The dry powder may be dissolved in sterile physiological salt solution in concentrations of 1000 to 5000 units per cubic centimeter for direct injection through a syringe.

"2. The dry powder may be dissolved in sterile saline or 5 per cent glucose solution in lower dilution (25–50 units per cubic centimeter) for constant intravenous therapy.

"b. For intramuscular injection

"1. The total volume of individual injections should be small, *i. e.*, 5000 units per cubic centimeter of physiological saline. It may also be given by constant intramuscular drip—120,000 units in 250 cc."

To the above it may be added that Sterile Isotonic Solution of Three Chlorides may also be employed as a solvent in the same manner as Sterile Isotonic Solution of Sodium Chloride.

In preparing solutions, the diluent transfer syringe and needle should be sterilized by boiling rather than by alcohol, since residual alcohol in the syringe assembly will exert an inactivating effect on the penicillin sodium. If alcohol is used for sterilization, the syringe assembly should be thoroughly dried or rinsed well with the diluent before being used. The same precautions obviously apply to the injection syringe and needle.

Scrupulous care should be exercised to maintain sterility in the preparation of solutions, since bacterial contamination may result in the production of destructive enzymes which will inactivate penicillin sodium. Solutions of penicillin sodium cannot be autoclaved without complete inactivation.

Solutions should preferably be freshly prepared and stored at 10° C. It is not recommended that solutions more than one to two weeks old, even if so stored, be used, because of possible significant loss of potency beyond that period.

Prolonging Effective Blood Level

Penicillin sodium is so rapidly excreted in the urine that following a single injection it is often impossible to detect it in the blood for a period longer than two to four hours. Since the maintenance of a therapeutic blood level is obviously essential for effective therapy, it is therefore necessary that, with aqueous solutions of penicillin sodium, repeated intramuscular or intravenous injections be made every three or four hours or that continuous infusion be employed.

Both procedures—repeated injections and continuous infusion—have practical disadvantages. These have led to studies on means to delay either the excretion of penicillin sodium or its absorption from the site of intramuscular or subcutaneous injection, in order to achieve therapeutically effective blood levels for prolonged periods of time.

Substances designed to delay urinary excretion —especially sodium para-aminohippurate solution[5]—have been administered with penicillin sodium with a resultant slowing of the excretion and consequent elevation and prolongation of plasma levels. The delayed urinary excretion resulting from the use of 6% sodium para-aminohippurate solution of penicillin sodium is explained as a competition between the two drugs for the same renal tubular excretory mechanism.

B. Intramuscular Suspensions:

The most effective means to date of obviating repeated injections, or continuous infusion, has been the employment of substances or a method to delay absorption of penicillin sodium from muscle or subcutaneous tissue. One obvious substance for this purpose is an oil, but simple peanut oil, sesame oil, cottonseed oil and castor oil suspensions of penicillin sodium or penicillin calcium have been shown to have but little effect on retarding absorption and on the duration of an effective level in the blood.[6] However, suspensions of penicillin calcium in peanut oil to which beeswax has been added have produced a significant prolongation of therapeutically effective blood levels lasting for seven to ten hours.[6]

Although studies on such suspensions are by no means complete, the following formula, based on the method of Romansky, is now receiving wide clinical evaluation:

Penicillin sodium.....	1,000,000 units
Bleached beeswax, U. S. P.....	0.2 Gm.
Purified peanut oil, *q. s.*.....	5 cc.

The formula does not lend itself to compounding in the average pharmacy because of the equipment necessary for preparing a sterile product. Ten 100,000 O.U. vials of penicillin sodium are required. The penicillin sodium "cake" is vigorously shaken to break it into pieces permitting exit from the orifice. The contents of nine of the vials are added to the tenth vial, or the contents of all the vials to a sterile 10-cc. bottle. The penicillin sodium is then further powdered by means of a sterile glass rod.

A sufficient quantity of beeswax to permit recovery of 0.2 Gm. is heated until clear and liquid, and filtered through six layers of gauze. About 5 Gm. of beeswax is necessary to obtain 0.2 Gm. of filtrate since the gauze layers retain a considerable amount. The peanut oil is warmed to 37° C. and filtered through a Seitz filter. Liquid beeswax, 0.2 Gm., is then added with a warm,

sterile pipette to 5 cc. of sterile peanut oil at 37° C.

This mixture is shaken well and added, by means of a sterile warm pipette, to the bottle containing the penicillin sodium. Five to ten sterile glass beads are then added, the vial stoppered with a sterile rubber stopper and shaken by hand until the penicillin sodium particles are well dispersed. Before administration, the suspension should be warmed to 37° C. and well shaken.

Evidence indicates that the use of penicillin calcium produces a suspension superior to one employing penicillin sodium. When available, penicillin calcium should be substituted for penicillin sodium in the above formula.

Stability tests on penicillin calcium in oil with beeswax show no deterioration when kept at refrigerator, room, and 37° C. temperatures for thirty to sixty-two days, according to Romansky. The suggested dosage of the above suspension is 1 cc. supplying 200,000 units of penicillin sodium. Clinical studies by Romansky have shown that 11 of 12 cases of gonococcic urethritis were cured by single injections ranging from 51,250 to 100,000 units and that an additional 53 patients also were cured by this method.

Vasoconstriction of the muscle site by a chilling technique using an ice bag two hours before and six to eight hours after an intramuscular injection has produced prolonged blood levels for six to twelve hours.[7]

2. Topical Forms

The employment of drugs topically offers the obvious advantage of an enormous concentration at the site of application. In the case of drugs of relatively high toxic potential, topical application has the additional advantage of avoiding subjecting the entire body to the toxic properties of the drug when the infection is circumscribed to a localized and accessible area. Depending upon the vehicle and the adjuncts to the application method employed, topical application has the further advantage of a "reservoir" effect, assuring continuous and prolonged contact of the drug with the infected lesion. Although penicillin sodium has an extremely low systemic toxicity, the advantages of high and prolonged local concentration and the convenience to the patient (not to mention the economical use of the drug) have encouraged clinical use of a number of topical forms of penicillin sodium.

A. Solutions:

Solutions of penicillin sodium topically applied were shown from the first to have no adverse effect upon tissue.[8] Clinical experience has been confirmatory and has demonstrated the high therapeutic efficacy of such treatment. The following, relative to topical application of solutions, is from the report by Dr. Chester S. Keefer.[4]

"For topical applications

"1. The powdered form of the sodium salt is irritating to wound surfaces and should not be used.

"2. Solutions in physiological salt solution with a concentration of 250 units per cubic centimeter are satisfactory. For resistant or more intense infections, this concentration may be increased to 500 units per cubic centimeter.

"In the treatment of meningitis, empyema and surface burns of limited extent, penicillin should be used topically, that is, injected directly in the subarachnoid space, into the pleural cavity, or applied locally in solution containing 250 units per cubic centimeter."

In addition to meningitis, empyema and surface burns of limited extent, solutions of penicillin sodium have been used topically with great success as drops for infections of the eye, such as blepharitis, acute and chronic conjunctivitis, and dacrocystitis; for infected chemical burns of the eye; as an irrigation or instillation for chronic wound sinuses, nasal sinus and middle ear infections; as a wet dressing for chronic ulcers and infected wounds of soft tissue and bone; and as aerosol solutions for inhalation therapy of lung infections.

Although clinical reports on topical use show a range of potency of solutions of penicillin sodium from 250 units to 10,000 units per cubic centimeter it is perhaps safe to assume that the potency most commonly employed currently is 1000 units per cubic centimeter. The same precautions for the preparation and storage of solutions for parenteral use apply to the preparation and storage of solutions for topical application.

Glycerin may be used as the solvent for ear drops, but potency loss in such a vehicle is greater than in aqueous vehicles.

The use of Gifford's buffer solutions for preparations of penicillin sodium for employment in the eye seems permissible, since no physical incompatibility exists with penicillin sodium. No data are yet available as to the rate of potency loss of

such buffered solutions of penicillin sodium, but extrapolation of the stability data from other electrolyte solutions seems warranted and stability therefore may be assumed to be comparable. Obviously the proportions of the two buffer solutions should be such as to assure the *p*H range of 6.3 to 6.8 within which penicillin sodium is most stable.

Neosynephrine and Privine Hydrochloride are apparently physically compatible with penicillin sodium,[9] and may therefore be employed in solutions for nasal spraying or nasal sinus irrigation when a vasoconstrictor effect is desired. The use of silver proteinate with penicillin sodium is not recommended because of possible inactivation by the silver ion.

B. Ointments:

Ointments of penicillin sodium have had rather wide and eminently successful clinical use, and doubtless this pharmaceutical form of penicillin will be the one pharmacists will have the greatest occasion to compound. Ointments have been used for a number of conditions, including pyrogenic infections of the skin and eye, soft tissue lesions, ulcers, and osteomyelitis. As in the case of solutions of penicillin sodium, the literature records a wide range of potency for ointments, but again current use indicates that 1000 units per gram is most commonly employed.

Wide latitude in so far as physical compatibility is concerned is permitted in the selection of a base for a penicillin sodium ointment, cream or jelly. However, differences in degree of retention of penicillin sodium potency do exist among various ointment bases.

As a general premise it can be stated that anhydrous bases have relatively less adverse effect upon the potency of penicillin sodium than do hydrous bases or bases containing free hydroxyl groups. Ointment bases containing phenolics or heavy metals have the most pronounced inactivating influence. The *p*H values of ointment bases also influence penicillin sodium potency, bases having a *p*H range of 6.3 to 6.8 being the most favorable. Determination of *p*H in ointment bases may be made by preparing a 1:10 dilution of the base with fresh distilled water and checking the *p*H with an indicator paper such as Nitrazine.*

As a matter of practical procedure, any type of ointment base, with the exception of those containing phenolics or heavy metals, can be used for compounding penicillin sodium ointments which will retain their potency for a period of two weeks if kept under conditions of refrigeration. The potency period of anhydrous base penicillin sodium ointments when kept at 10° C. appreciably exceeds two weeks.

As a measure of conservatism, however, and in recognition of other factors which influence potency, large quantities should not be prepared for stock, but only prepared freshly for each prescription or at the most for each day's requirements, and then only filled in dispensing unit containers and not in bulk containers.

It is most important in the preparation of penicillin sodium ointments that where heat is employed in compounding or in sterilization of the base, the base should be cooled at least to 37° C. *before* the penicillin sodium is incorporated. At temperatures exceeding 37° C. loss of penicillin sodium potency will occur very rapidly.

The penicillin sodium should be added to the warm base as a solution in sterile distilled water, using a minimum of water (2 to 3 cc.) for solution, thoroughly mixed and the ointment then *rapidly* cooled. Incorporation of the penicillin sodium solution in the base should be done with care to avoid concomitant incorporation of air. Since certain heavy metals have an adverse influence on penicillin sodium potency, it is preferable that ointment tubes or tins be interiorly wax coated, or that glass jars be employed as the dispensing unit. Ointments compounded from hygroscopic bases should be dispensed in tubes rather than in tins or jars.

Only small quantities should be dispensed, especially if the container is a tin or jar, since each use of the ointment unavoidably introduces bacterial contamination which may decrease the potency of the penicillin sodium.

Experiments have disclosed that penicillin calcium possesses properties admirably suited to the preparation of fine ointments. When penicillin calcium is commercially available, it is recommended as the preferred salt of penicillin for ointment purposes.

General Purpose Ointments

The following formulas for ointments of penicillin sodium are listed to suggest types which may be employed on prescription. Most of them are representative of formulas which have appeared in the medical literature.

* See THIS JOURNAL, 6: 8, 1945 (annotations), for sources of indicator papers.

White petrolatum is a good base for a penicillin sodium ointment in that its anhydrous character provides a measure of potency retention not exceeded by any base yet studied. In preparing the ointment, the pharmacist may weigh the contents of a vial of penicillin sodium, 100,000 units, and easily calculate the unitage per milligram. Incorporation of the required number of units of penicillin sodium in white petrolatum can be made by simple art, either by direct incorporation or by first preparing a paste with liquid petrolatum.

Mixtures of wool fat or hydrous wool fat and white petrolatum can be employed by simple art to prepare ointments of penicillin sodium, although if water is used in its compounding, as frequently practiced, a factor contributing to potency loss is introduced which is not possessed by a white petrolatum base ointment. The adverse effect of water upon penicillin sodium potency is obviously present when hydrous wool fat is used as a base component. If white wax is to be a component of the base, the precautions relative to the potency relationship of heat and air to penicillin sodium should be observed.

Here are three "washable" oil-in-water formulas from the literature. The following formula, which produces an ointment of creamy consistency, is modified slightly from that reported by Grainger.[10]

Lanette wax SX*..... 12 parts
Peanut oil..... 25 parts
Distilled water, *q. s.*.....100 parts
Penicillin sodium, *q. s.* to prescribed unitage

Melt the wax with the oil on a water bath (60–70° C.) with stirring, and add to it the distilled water heated to the same temperature. Cool to 37° C., rapidly stir in the penicillin sodium solution and *quickly* cool.

The following formula is modified slightly from that reported by Barron and Mansfield.[11]

Lanette wax SX* }
Soft petrolatum } equal parts
Distilled water }
Penicillin, *q. s.* to prescribed unitage per gram

It is suggested that this formula be compounded in the same manner as the preceding one.

The following formula which has been extensively employed for burns in England was obtained from private sources.

Lanette wax SX..... 10 parts
Castor oil..... 25 parts
Distilled water, *q. s.*.....100 parts
Penicillin, *q. s.* to 200 units per gram

It is also suggested that this formula be compounded in the same manner as the preceding two.

Ophthalmic Ointments

The following formula was reported by Bellows.[12]

Simple ointment, U. S. P.
Penicillin (sodium), *q. s.* to 2500 units per gram

A suggested method of preparation is:

Levigate the penicillin sodium with Simple Ointment to a smooth consistency; or if the Simple Ointment is to be compounded, 2 to 3 cc. distilled water solution of penicillin sodium may be rapidly incorporated after the base has been cooled to 37° C. and the ointment then *quickly* cooled.

According to Bellows, experiments with rabbits on the penetration of penicillin sodium into the eye showed Simple Ointment U. S. P. to be the most satisfactory base, an average concentration of 0.42 unit penicillin appearing in the aqueous humor after application of the ointment to the conjunctiva for one hour. Other vehicles tested in order of penetrability of penicillin sodium were: a vanishing type stearate base, an oil-in-water emulsion and a lubricating jelly. Injury to the cornea was observed when a vanishing type base was used.

The following ointment was reported by Keyes.[13]

Petrolatum or hydrous wool fat.. 25 parts
Cold cream..... 75 parts
Penicillin sodium, *q. s.* to 250 to 500 units per gram

A suggested method of preparation is:

Levigate the penicillin sodium with the petrolatum (or the hydrous wool fat, if it is selected in preference) to a smooth paste and incorporate the mixture with the cold cream.

* Lanette wax SX is a British proprietary. A replacement for this ingredient is available from the R. F. Revson Co., 144 West 18th St., New York 11. Lanette wax is briefly described (*Pharm. J.*, 145: 85, 1940) as "a mixture of stearyl and cetyl alcohols with a proportion of their phosphated esters. At ordinary temperatures, it is a white hard solid melting at a comparatively low temperature, is miscible with oils, waxes and fats and forms stable neutral emulsions with aqueous media of the oil-in-water type." It is reported that this product can be approximated by combining 9 parts of cetyl alcohol flakes with 1 part of sodium lauryl sulfate.

According to Keyes this penicillin sodium ointment (it is not stated which formula is referred to—that using petrolatum or that using hydrous wool fat) showed appreciable penicillin activity after six months in a refrigerator, but diffusion activity lessens significantly after twenty-five days at room temperature, although one specimen was still potent after forty-five days at room temperature.

In addition to the foregoing ointment bases, the proprietary ointment bases Aquaphor and Hydrosorb may be employed.

We are indebted to several hospital pharmacists for formulas for ointments of penicillin sodium in use at their hospitals. These formulas and their sources follow:

Union Memorial Hospital, Baltimore
Anthony P. Mentis, chief pharmacist

Two hundred grams of Hydrosorb (Abbott) is autoclaved in a covered Pyrex beaker for eight minutes at 15 lb. pressure. Just before the Hydrosorb reaches room temperature and while it is still liquid (autoclaving liquefies it), a solution of penicillin, made by dissolving 100,000 units in not more than 2 or 3 cc. of sterile water, is rapidly stirred in and thoroughly mixed. This is then poured into ordinary ointment tins and placed in the refrigerator until used. Aseptic technique is observed. The above quantities give a final strength of 500 units per gram.

New York Eye and Ear Infirmary
Andrew H. Witze, pharmacist

PENICILLIN OPHTHALMIC OINTMENT

Penicillin	100,000 units
Mineral oil	10 ʒ
Lanolin, anhydrous	25 ʒ
White petrolatum, *q. s.*	100 ʒ

Levigate the penicillin sodium with the mineral oil, and incorporate the mixture with the anhydrous lanolin and the white petrolatum.

Drug Service Dept., College of Pharmacy,
State University of Iowa
Louis C. Zopf, professor

"We have had occasion to prepare an ointment of penicillin. For this preparation we found it convenient to dissolve the penicillin in the smallest amount of water possible. I believe I am safe in saying that, in order to dissolve the penicillin and rinse the vial, not more than 2 cc. of water was used. This concentrated aqueous solution of penicillin was then incorporated into an ointment base known in our laboratory as aquabase. Aquabase is composed of 3% cholestrin, 3% cottonseed oil and 94% white petrolatum. The concentration of the penicillin ointment was adjusted so that 100 Gm. of the finished product represented 100,000 units of penicillin. This particular ointment was used in our Ophthalmology Department for definitely proved streptococcus and staphylococcus conjunctivitis. The physicians, while they did not run a control, are confident that the ointment remains potent for a period of three weeks at least. You understand, of course, that our penicillin ointment was kept refrigerated at all times."

C. Pastilles:

Penicillin sodium pastilles have been used topically for infections of the mouth.[14] The infections treated included acute ulcerative gingivostomatitis (Vincent's type) and acute hemolytic streptococcal tonsillitis; the pastilles were also used for prophylaxis against infection in trauma of the mouth, such as that following tooth extraction. It is reported that results were uniformly excellent, both in the control and prevention of infection, except in the treatment of chronic throat carriers of hemolytic streptococci.

The formula and method of preparing such pastilles is as follows:[15]

Gelatin powder	440 gr.
Nipagin M*	2.5 gr.
Simple syrup	2 fʒ
Distilled water	2 fʒ
Penicillin sodium, *q. s.*	250–500 units per pastille

(NOTE: The above formula will make 200 pastilles of a 1 sq. cm. area and a 0.5 cm. thickness.)

Dissolve the Nipagin M* in boiling distilled water, add the simple syrup, stir in the gelatin and when well mixed, pour into a mold lubricated with persic oil. Stir the melted base until the temperature reaches 43° C. and then quickly add the required amount of penicillin sodium dissolved in a minimum of distilled water. Stir with a glass rod to distribute the penicillin sodium evenly, cool, and cut into pastilles.

Pastilles so prepared are reported to provide an effective and sustained salivary concentration of penicillin for fifteen to thirty minutes.

Infected wounds of the mouth have also been

* Nipagin M is a British proprietary name for methyl parahydroxybenzoate N. F. VII.

successfully treated with cone-shaped tablets consisting of a mixture of penicillin sodium 500 units and sulfanilamide.[16] Such tablets have been used for prophylaxis after tooth extraction, in dry socket, cellulitis, buccinator muscle infections and osteomyelitis.

D. Powders:

Powders of penicillin sodium have been used occasionally for dusting on surface wounds and for insufflation into body cavities. A simple triturate of sulfanilamide or sulfathiazole with 5% light magnesium oxide and a sufficient quantity of penicillin sodium to yield a potency of 2000 to 5000 units per gram has been used with success in preventing and controlling infection.[17] In addition to a powder of the above formula, a powder mixture of dried human serum and a quantity of penicillin sodium to provide 3000–4000 units per gram has also been employed.[18]

A somewhat comparable mixture of penicillin sodium and dehydrated plasma has also been used as a powder spray for the local treatment of wounds.[19] The formula consisted of 10,000 or 20,000 units of penicillin sodium mixed with 0.2 Gm. dehydrated plasma. It is reported that the powder goes immediately into solution upon contact with the wound surface, and that *in vitro* studies indicate that the penicillin is not inactivated by the dehydrated plasma.

E. Suppositories:

Because penicillin sodium is speedily inactivated by the enzyme penicillinase, produced by *E. coli*, the employment of it in suppositories, or in any form intended for rectal administration, is irrational and should be discouraged.

3. Oral Forms

Long the goal of research has been the development of an effective and relatively economical form of penicillin for oral administration. Early studies had demonstrated that penicillin sodium was rapidly destroyed by the hydrochloric acid of the stomach and was followed by poor absorption.[1, 8] Studies[1] had also indicated that penicillin sodium could be effectively absorbed in the duodenum. Thus the problem of an effective oral dosage form was simply [*sic!*] one of providing means to protect penicillin from the stomach content of hydrochloric acid, or to buffer the hydrochloric acid in the stomach so that the *p*H range is attained in which penicillin is not inactivated.

The obvious procedures to attain these ends are the use of alkaline substances for their buffering effect or enteric coated tablets or capsules to protect the penicillin from the acid during its passage through the stomach. The use of an alkali such as sodium bicarbonate has given indifferent results.[1]

Results with enteric coated tablets for oral administration have been stated to be discouraging.[8, 20] Unpublished studies have also disclosed that the administration of enteric coated capsules of penicillin is followed by poor absorption and that there are indications that penicillin, to be absorbed effectively, must be available in the duodenum or the jejunum.

Recently it was reported that capsules of a cottonseed oil suspension of penicillin, administered as a single dose of 90,000 units, maintained a fairly uniform therapeutic blood level of between 0.03 and 0.06 unit of penicillin per cubic centimeter for at least four hours.[20] Even though less highly refined penicillin may be adaptable for oral dosage forms for systemic effect, their cost for effective therapy will doubtless be relatively much higher than that of injection forms. The road to an economical effective oral penicillin is still a long hard one.

Summary

1. Although greatly restricted in scope and detail, information on the pharmacy of penicillin is presented which, it is hoped, will be of some value to pharmacists in the compounding of penicillin pharmaceutical forms.

2. Penicillin is an extremely sensitive substance and its nature must be understood if its invaluable antibiotic effects are to be best realized.

3. Penicillin lends itself, within limitations imposed by its sensitive nature, to the compounding of such pharmaceutical forms as solutions, suspensions, ointments, powders, pastilles, tablets and capsules.

4. The pharmacist must recognize those limitations and confine their influence by full observance of the conditions of storage, temperature, moisture, *p*H and aseptic technique required for optimum activity of penicillin and the penicillin pharmaceutical forms he compounds.

In one of the first reports on penicillin it was stated that penicillin should not be entrusted to the pharmacist but that preparation of its solu-

tions (the only pharmaceutical form then employed) should be made by the bacteriologist. The absurdity of this challenge to the competence of the modern pharmacist need not be dignified by defense. The pharmacist is competent not only to be entrusted with the intelligent preparation of pharmaceutical forms, but also to be entrusted with an equally great concomitant responsibility—the responsibility for doing all in his power to prevent the misuse of a drug which, although the most powerful antibacterial substance known, is still not a "cure-all."

Grateful acknowledgement is made to the many pharmacists who, although severely handicapped by insufficient supplies of penicillin to study adequately the compounding of penicillin pharmaceutical forms, have nonetheless contributed greatly to this article by their splendid suggestions.

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PYROGENS IN PENICILLIN MAY BE DUE TO FILM ON GLASS

Fever-producing pyrogens, found in an occasional lot of penicillin, may be largely due to improper cleansing of glassware rather than improper production methods, according to studies by the Food and Drug Administration reported in the April *Scientific Edition* of THIS JOURNAL.

In a large percentage of cases of pyrogen-contaminated products, it was found that the bulk solutions were free from pyrogen but the glass ampuls themselves contained a film of thermogenic material on the inner surface. Ordinary washing procedures with sterilization by autoclaving or dry heat are not sufficient to destroy or remove the pyrogen in this film.

It was found that a temperature of at least 250° C. for forty minutes is advisable to assure destruction of pyrogens on glassware. Under commercial conditions 200° C. for two hours has been found satisfactory.

To obtain accurate results in pyrogen tests, all equipment, such as pipettes and syringes, must be similarly heated.

In the production of penicillin itself, it is inevitable that pyrogens will develop due to the lysis of mold mycelia and spores. These are at least partially removed by the extraction process and are later completely removed by adsorptive filtration through asbestos pads (Ertel or Seitz). In experiments with this filtration process, the FDA workers found that it is easier to free impure penicillin from pyrogens than to free more potent samples from the contaminant.

There is a definite relationship, they conclude, between the volume of penicillin which may be filtered free from pyrogen through a given filter unit and the purity of the product.

Those conducting the study were Henry Welch, Clifford W. Price, Velma L. Chandler and Albert C. Hunter.

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HUNDREDS of papers have been published in professional periodicals on penicillin investigations. Much of the information pertinent to present-day practice is reviewed in the series of papers published in this issue of the JOURNAL. Extensive references to source material are included to aid the pharmacist and physician in pursuing specific questions further.

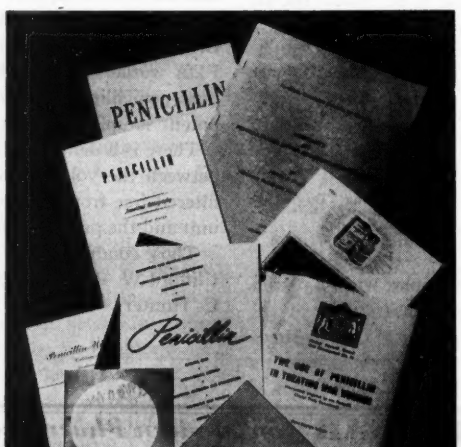
If reprints are not available from authors, back copies of professional publications containing papers of particular interest to you or your physicians can usually be obtained. The book service of THIS JOURNAL will supply on request the address and single-copy price of any journal needed.

In addition, booklets and pamphlets on penicillin are listed below. Many commercial laboratories have issued publications containing professional information, and wherever a price is not given the publication will be sent without charge on request.

ABSTRACTS ON PENICILLIN AND OTHER ANTIBIOTIC SUBSTANCES—Muriel E. Whalley—*National Research Council of Canada*, Ottawa, Ontario, 71 pp., $1. Contains 185 abstracts largely compiled from American Chemical Abstracts. Indexed by antibiotic substances.

PENICILLIN 1929–1943 (Special Issue of British Medical Bulletin, No. 1, Vol. 2, 1944), *British Publications Co.*, 726 Wabash Bldg., Pittsburgh, Pa., 28 pp., $1.25. Contains special papers by Florey, Chain, Fleming and other British leaders in penicillin investigations. Reviews selected papers from the British literature starting with Prof. Fleming's first report on his discovery of penicillin in 1929.

USE OF PENICILLIN IN TREATING WAR WOUNDS—British Medical Research Council—*His Majesty's Stationery Office*, British Information Service, 30 Rockefeller Plaza, N. Y., 16 pp., $0.10. A memorandum from the Penicillin Clinical Trials Committee discussing dispensing, administration, use in various types of wounds and clinical laboratory procedures.



PENICILLIN AND THE PRESENT-DAY CONCEPT OF ITS CLINICAL APPLICABILITY—*Commercial Solvents Corp.*, Pharmaceutical Div., 17 E. 42nd St., New York 17, N. Y., 71 pp. Well-prepared booklet. Includes therapeutic reference table and subject index.

A REVIEW OF THE PRESENT INFORMATION CONCERNING PENICILLIN—*Abbott Laboratories*, Advertising Service Dept. North Chicago, Ill., 109 pp. Illustrated. Summarizes work of leading investigators, including bacterial studies, comparisons with chemotherapeutic agents, production, properties and pharmacology. Nearly half of the text is devoted to clinical use and administration.

PENICILLIN ANNOTATED BIBLIOGRAPHY—*Winthrop, Chemical Co.*, Library, 170 Varick St., New York 13, N. Y. First Series contains references 1 through 81, with subject index. Second Series contains references 82 through 260 with supplemental ref-

erences and cumulative author and subject indexes.

PENICILLIN REPORTER—*Commercial Solvents Corp.*, Pharmaceutical Div., 17 E. 42nd St., New York 17, N. Y. Bimonthly pamphlet containing abstracts of reports on penicillin investigations. (Limited number of back copies available.)

PENICILLIN SODIUM—*E. R. Squibb & Sons*, 745 Fifth Ave., New York 22, N. Y., 14 pp. Summary of properties, indications, dosage and administration. Includes table of sensitive and nonsensitive organisms.

PENICILLIN QUIZ—*Cheplin Biological Laboratories*, Syracuse, N. Y., 13 pp. Basic information in question and answer form.

SELECTED LITERATURE REFERENCES ON PENICILLIN—*Hoffmann-La Roche, Inc.*, Roche Park, Nutley, N. J., 10 pp. One-hundred thirty references listed by author.

PENICILLIN—*Eli Lilly and Co.*, Indianapolis 6, Ind., 22 pp. Concisely covers history, chemistry, pharmacology, clinical use and administration.

PENICILLIN—*Winthrop Chemical Co.*, New York 13, N. Y., 22 pp. Properties, pharmacology, clinical considerations, indications and contraindications, administration and dosage.

THE STORY OF PENICILLIN—*Merck & Co.*, Rahway, N. J., 30 pp. Well-illustrated; emphasizes clinical aspects.

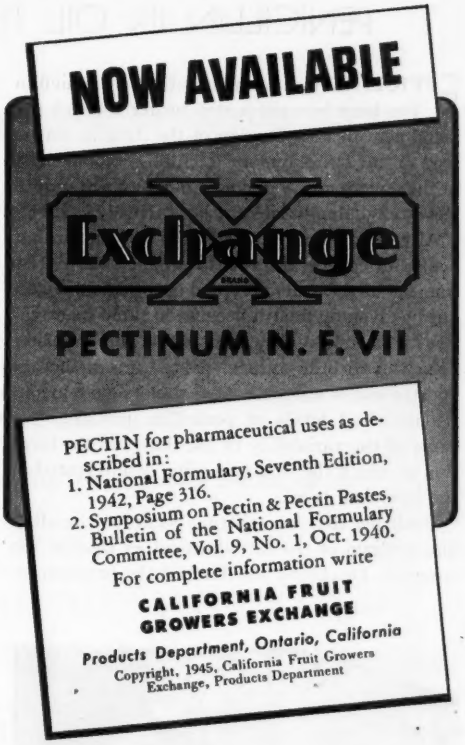
PENICILLIN—*Parke, Davis & Co.*, Advertising Dept., Detroit 32, Mich., 14 pp. Summarizes clinical aspects, standardization, tolerance and toxicity, pharmacology and physical properties.

PENICILLIN: Its Development and Therapeutic Application—*Schenley Laboratories*, 350 Fifth Ave., New York 1, N. Y., 23 pp.

PENICILLIN—*Cutter Laboratories*, 4th and Parker Sts., Berkeley, Calif., 19 pp.

SYNERGISM OF PENICILLIN WITH OTHER DRUGS STUDIED

Synergism of penicillin and sulfapyridine in subtherapeutic doses is confirmed and a similar effect demonstrated in combinations of penicillin with sulfanilamide and with *p*-nitrobenzoic acid by tests on streptococcic infections of mice, according to Drs. G. Soo-Hoo and R. J. Schnitzer of Hoffmann-La Roche Research Laboratories (*Arch. Biochem.*, 5:99, 1944).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Tests on *p*-aminobenzoic acid, chosen because it potentiates penicillin activity *in vitro*, failed to show synergism *in vivo*. There was likewise no synergistic action when penicillin was combined with antistreptococcal agents of the acridine group or with the gold compound, myochrysine.

In view of the marked differences in synergistic action, further studies are being made to determine the mechanism involved.

The synergistic effect of the combined dosage of penicillin and sulfonamide was "more or less dependent on the dose of the sulfonamide."

The authors are inclined to believe that the action is facilitated by the difference in the mechanism by which the sulfonamides and penicillin act. Failure to successfully combine acridine dyestuffs and gold compounds with penicillin may be due to a mechanism of action different from the sulfonamides.

PENICILLIN IN OIL TESTED FOR ORAL USE

EFFICIENT oral administration of penicillin has been brought a step nearer through development of a suspension of the drug in cotton seed oil by Dr. Raymond L. Libby, biophysicist in the American Cyanamid Company's Stamford Research Laboratories (*Science*, 101:178, 1945).

Although several investigators had shown that penicillin can be absorbed from the small intestine, it was generally accepted that penicillin cannot be given by mouth because of rapid inactivation by gastric acidity. Attempts to administer penicillin with an antacid proved unsatisfactory and the use of enteric coated tablets gave inconsistent blood levels of penicillin, probably because of the variability in the time and the location at which the enteric coating disintegrated in the intestinal tract.

Realizing that fat splitting occurs in the alkaline medium of the intestines rather than in the stomach, Dr. Libby approached the problem by suspending penicillin salts in various fats and oils.

DR. RAYMOND L. LIBBY at work in the Stamford Laboratory of the American Cyanamid Company, where he developed a form of penicillin suitable for oral administration. Plans are already under way for production by Lederle Laboratories.

The highest concentration was obtained in cotton seed oil. For clinical trial, suspensions of the sodium and calcium salts of penicillin (150 to 300 units per milligram) were prepared and dispensed in gelatin capsules containing either 10,000, 25,000 or 50,000 units per capsule.

Results of an unspecified number of tests on dogs and man led to the conclusion that "a single oral dose of 90,000 units of penicillin in oil will maintain a fairly uniform therapeutic blood level for a period of at least four hours." Compared with the blood levels obtained after the intramuscular injection of 20,000 units of an aqueous solution of penicillin "it is apparent that 2 or 3 or possibly more injections would be required to maintain a comparable blood concentration over the same period."

Even with the oral administration of penicillin in oil a portion is inactivated, probably by the gastric acidity.

"Although these preliminary results indicate that a greater amount of penicillin might be required if administered orally than if by intramuscular injection," Dr. Libby states, "this increased use of penicillin will probably be offset by several factors. In this regard certainly the greater ease of administration both from the point of view of the doctor as well as the patient must be considered. Furthermore, for oral use less highly refined penicillin should be entirely satisfactory, thus simplifying the present procedures for the production of suitable material."

TEST FOR PENICILLIN TITER IN FLUIDS

A procedure for testing body fluids for penicillin titer, which is claimed to be much simpler than other methods commonly used, is reported by Dr. Jean V. Cooke of the Washington University School of Medicine, St. Louis (*J. Am. Med. Assoc.*, 127: 445, 1945).

In the test, the smallest amount of fluid is determined which will completely inhibit the growth of a standard strain of *Staphlococcus aureus* on a plain agar Petri plate. The materials and technique will be familiar to clinical technicians.

The procedure can also be used to determine variations in the penicillin sensitivity of various strains of infectious bacteria.

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RESEARCH ON ESTERS MAY YIELD BETTER W/O BASE

Of a series of cholesteryl esters studied, cholesteryl stearate most effectively increased the emulsifying efficiency of a solution of cholesterol in white petrolatum. This fact is brought out in a report in the *Scientific Edition* of THIS JOURNAL (34:33, 1945) by E. L. Cataline and S. F. Jeffries of the University of Michigan College of Pharmacy and Frank Reinish, pharmacist at the Mercer Hospital, Trenton, N. J.

The report covers part of a series of investigations to secure information on why some cholesteryl esters have the ability of enhancing the emulsifying efficiency of cholesterol-petrolatum mixtures more than threefold.

To a limited extent there appears to be a correlation between increased molecular weight of an ester and increased power as a booster of emulsifying efficiency. This theory did not hold, however, for cholesteryl stearate which surpassed other esters of considerably higher molecular weight.

In extending their study to cetyl esters, the pharmacists found that in only one instance, dicetyl adipate, was the cetyl ester as satisfactory in increasing the efficiency of cholesterol-white petrolatum mixture as the corresponding cholesteryl ester.

There was some evidence to indicate that the addition of a hydroxy group to an ester enhances its action. Experimentally it was shown that the most efficient ester, cholesteryl stearate, was still more efficient when an hydroxyl group was added. The possibilities suggested by this discovery will be explored further.

From the investigations it is hoped that an inexpensive, stable, water-absorbent ointment base can be developed, which would be devoid of the objectionable features of lanolin-containing bases.

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